The globally distributed genus *Alexandrium*: multifaceted roles in marine ecosystems and impacts on human health

Donald M. Anderson[1], Tilman J.Alpermann[2], Allan D. Cembella[3], Yves Collos[4], Estelle Masseret[4], and Marina Montresor[5]

[1] Woods Hole Oceanographic Institution, MS # 32, 266 Woods Hole Road, Woods Hole MA 02543; email: danderson@whoi.edu; 508 289 2351 (Corresponding author)

[2] LOEWE Biodiversity and Climate Research Centre (BiK-F), Senckenberg Research Institute, Senckenberganlage 25, 60325 Frankfurt a.M., Germany; email: Tilman.Alpermann@senckenberg.de

[3] Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570, Bremerhaven, Germany; email Allan.Cembella@awi.de

[4] Ecologie des systèmes marins côtiers, UMR 5119, UM2, CNRS, IRD, Ifremer, UM1, Université Montpellier 2, CC 093, 34095 Montpellier, France; email: yves.collos@univ-montp2.fr ; estelle.masseret@univ-montp2.fr

[5] Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy; email marina.montresor@szn.it

Abstract

The dinoflagellate genus *Alexandrium* is one of the major harmful algal bloom (HAB) genera with respect to the diversity, magnitude and consequences of blooms. The ability of *Alexandrium* to colonize multiple habitats and to persist over large regions through time is testimony to the adaptability and resilience of this group of species. Three different families of toxins, as well as an as yet incompletely characterized suite of allelochemicals are produced among *Alexandrium* species. Nutritional strategies are equally diverse, including the ability to utilize a range of inorganic and organic nutrient sources, and feeding by ingestion of other organisms. Many *Alexandrium* species have complex life histories that include sexuality and often, but not always, cyst formation, which is characteristic of a meroplanktonic life strategy and offers considerable ecological advantages. Due to the public health and ecosystem impacts of *Alexandrium* blooms, the genus has been extensively studied, and there exists a broad knowledge base that ranges from taxonomy and phylogeny through genomics and toxin biosynthesis to bloom dynamics and modeling. Here we present a review of the genus *Alexandrium*, focusing on the major toxic and otherwise harmful species.

Keywords: Alexandrium, harmful algal blooms, HAB, biotoxins, public health, global dispersion

1 Introduction

Among the genera responsible for harmful algal blooms (HABs), the genus

Alexandrium is certainly one of the most important in terms of the severity, diversity, and distribution of bloom impacts. Of the more than 30 morphologically defined species in this genus, at least half are known to be toxic or have otherwise harmful effects (Table 1). One unique feature of this genus is that three different families of known toxins are produced among species within it – saxitoxins, spirolides, and goniodomins. This toxigenic diversity is not found in any other HAB genus.

The most significant of these toxins in terms of impacts are the saxitoxins, responsible for outbreaks of paralytic shellfish poisoning (PSP), the most widespread of the HAB-related shellfish poisoning syndromes. The impacts of PSP outbreaks include human intoxications and death from contaminated shellfish or fish, loss of wild and cultured seafood resources, impairment of tourism and recreational activities, alterations of marine trophic structure, and death of marine mammals, fish, and seabirds. The macrocyclic imine spirolides, thus far known only from *A. ostenfeldii* (Cembella et al., 2001) and possibly *A. peruvianum* (as listed in the IOC taxonomy database; Moestrup et al., 2011), are potent fast-acting neurotoxins when administered intraperitoneally into laboratory rodents. No human cases of shellfish poisoning from spirolides have been documented, however, and subsequent toxicological investigations have not justified their inclusion in regulatory regimes for seafood toxicity. The goniodomins produced by *Alexandrium monilatum* and *A. hiranoi* (formerly *Goniodoma pseudogonyaulax*; Hsia et al., 2005) cause paralysis and mortality in finfish. They are not linked to human illness, and are not a major problem on a global scale.

Many of the species within *Alexandrium* have been well studied scientifically, leading to major advances in our understanding of their biogeography, genetics,

toxinology, physiology, ecology and management. Here we present a review of the *Alexandrium* genus, focusing on the major toxic or harmful species. Space limitations preclude a comprehensive review of all aspects of all species in this large genus. Instead, examples are provided of research results and observations that are broadly informative or that are indicative of approaches leading to improved understanding of other species. One focus is on autecological features that underlie many *Alexandrium* blooms, based predominantly on the small number of species that have been well studied in the laboratory and the field. Another key issue is life cycle transformations and their quantitative effect on bloom dynamics because in this specific area *Alexandrium* blooms have been especially well-characterized and differences from other HAB taxa become apparent. Another unique attribute is that *Alexandrium* genetics have received considerable attention, both from a phylogenetic perspective, and in terms of identifying genes and gene expression patterns for critical pathways, such as that for toxin production.

2 *Alexandrium* Species

2.1 Taxonomy and phylogeny of *Alexandrium*

The genus *Alexandrium* was formally established with the description of its type species *A. minutum* Halim (Halim, 1960), a small-sized dinoflagellate that produced a 'red tide' in the harbor of Alexandria in Egypt. This genus now includes 31 species (Table 1), many of them originally described under a different genus name (as *Gonyaulax, Protogonyaulax, Gessnerium, Goniodoma, Pyrodinium*). This fact reflects the intricate taxonomic history of these species, as well as subjective interpretations of

89 the stability and importance of particular morphological characters for the delineation of
90 genera and species. From the morphological point of view, the species now included in
91 the genus *Alexandrium* share a Kofoidean plate pattern of APC (apical pore complex), 4',
92 6'', 5''', 2'''', 6C, 9-10S (Balech, 1995). Cells are relatively featureless when observed
93 by light microscopy but minor morphological characters become visible after staining
94 and dissection of thecal plates and/or after examination by scanning electron microscopy.
95 Morphological characters for species identification are: cell size, shape, chain formation,
96 ornamentation of the theca, cingular and sulcal excavation, sulcal lists, shape of APC, 1',
97 6'' and some sulcal plates, such as S.p., S.a., S.s.a. A detailed illustration, description and
98 discussion of the various species are presented in the monograph by Balech (1995).
99 Resting cysts have been described for many *Alexandrium* species and, with the exception
100 of *A. pseudogonyaulax*, which forms cysts with a distinct paratabulation (Montresor,
101 1995), they have a smooth wall and a round, oval, or elliptical shape (Matsuoka and
102 Fukuyo, 2003).

103 The genus *Alexandrium* is subdivided into two subgenera: *Alexandrium sensu*
104 *strictu* (where the 1' plate is connected to the APC) and *Gessnerium* (where the 1' plate is
105 not connected to the APC; Table 1). When he established the two sub-genera, Balech
106 (1995) already recognized that *Gessnerium* is a heterogeneous group composed of
107 morphologically distinct species.

108 Molecular phylogenetic analyses – mostly carried out on genes of the ribosomal
109 RNA (rDNA) in unicellular eukaryotes (including dinoflagellates) – confirmed that
110 *Alexandrium* belongs to the Gonyaulacales (e.g., Saldarriaga et al., 2004). Sequence
111 analyses of members of the genus *Alexandrium* support the taxonomic distinction from

112 other gonyaulacoid genera by unequivocally corroborating the monophyletic nature of the
113 genus (e.g., Usup et al., 2002; John et al., 2003b; Saldarriaga et al., 2004). Only a single
114 publication on *Alexandrium* phylogeny suggested a paraphyletic nature of the genus
115 because large ribosomal subunit (LSU) rDNA sequences of *Pyrodinium bahamense*
116 diverged from within a clade otherwise exclusively composed of *Alexandrium* species
117 (Leaw et al., 2005). Though these findings have not been explicitly contested prior to the
118 present review, paraphyly of *Alexandrium* must be doubted as some inconsistencies with
119 previous studies can be observed in the phylogenetic tree presented by Leaw et al. (2005).
120 Moreover, neither the maximum likelihood- nor the maximum parsimony- based analysis
121 in Leaw et al. (2005; Fig. 3 (A) and (B), respectively) give statistical support for
122 paraphyly of *Alexandrium*. Our phylogenetic analyses, including LSU rDNA sequences
123 of the majority of the currently recognized *Alexandrium* species and sequences of *P.*
124 *bahamense* used by Leaw et al. (2005), support the reciprocal monophyly of the two
125 genera (Fig. 1). The close phylogenetic proximity of *Pyrodinium* to *Alexandrium* remains
126 uncontested, as this is consistent with the prior taxonomic assignment to *Pyrodinium* of
127 several species that now belong to *Alexandrium* (Table 1).

128 The phylogenetic analyses conducted for this review (Fig. 1) identify several well-
129 supported clades in the genus *Alexandrium*, although DNA sequences are not available
130 for all members of the two subgenera (e.g., the *Gessnerium* group species *A. balechii* and
131 *A. foedum*). In any case, as reciprocal monophyly is not found for the subgenera
132 *Alexandrium* and *Gessnerium*, molecular phylogenies do not fully corroborate this
133 taxonomic division of the genus as proposed by Balech (1995). In fact, whereas *A.*
134 *hiranoi*, *A. monilatum*, *A. pseudogonyaulax*, *A. saotanum* and *A. taylori* consistently form

a well-supported clade that diverges early from all species of the subgenus *Alexandrium*, two species of the subgenus *Gessnerium* (*A. margalefi* and *A. insuetum*) do not fall into this clade (Hong et al., 2008; Touzet et al., 2008a,b; Fig. 1). *Alexandrium margalefi* either shows affinity to this clade with low support (Kim et al., 2005) or relates with only weak to moderate support to a clade including *A. minutum, A. angustitabulatum, A. tamutum* and the *A. ostenfeldii/A. peruvianum* species complex, where it branches off early (John et al. 2003b; Kim et al., 2005; Touzet et al., 2008a). *Alexandrium insuetum* is instead consistently placed within this latter clade (e.g., Hansen et al., 2003; Leaw et al., 2005; Penna et al., 2008; Kremp et al., 2009). While the subgeneric classification by morphological criteria for the majority of *Gessnerium* species seems evolutionarily meaningful, at least in *A. insuetum*, plate characteristics have been suggested to result from convergent evolution (Touzet et al., 2008a).

A close phylogenetic relationship is confirmed for the morphologically defined species *A. tamarense*, *A. fundyense*, *A. catenella*, *A. affine*, *A. tamiyavanichi*, *A. cohorticula*, *A. tropicale* and *A. fraterculus*. The first three and the latter four species form distinct clusters, respectively. The branching pattern of *A. affine* sequences and these two clades differs depending upon the phylogenetic approach and sequences used for the analysis (e.g., Touzet et al., 2008a,b,) and statistical support for either of the possible branching patterns is low. All species of this larger clade are potentially harmful due to their capacity for PSP toxin production and have been the focus of many studies that included morphological and genetic characterization of strains of different geographical origin. These studies highlighted the existence of species-complexes, such

157 as the *A. tamarense*/*catenella*/*fundyense* group, i.e. genetically distinct clusters of strains
158 sharing very similar morphological features.

159 The three morphospecies *A. tamarense, A. catenella* and *A. fundyense* were
160 distinguished based on different combinations of two main characters: the capability to
161 form chains and the presence/absence of a ventral pore between plates 1' and 4'. Due to
162 the lack of match and inconsistencies between morphological discrimination characters,
163 toxicity, and genetic resolution among the three species, they were thus grouped within
164 the '*A. tamarense* species complex' (Anderson et al., 1994; Scholin et al., 1994). Five
165 ribotypes were identified and named after the geographical origin of the strains: North
166 American, Western European, Temperate Asian, Tasmanian, and Tropical Asian (Scholin
167 et al., 1994). In a subsequent study, a new non-toxic endemic Mediterranean ribotype of
168 *A. tamarense* was described and phylogenetic analyses showed that the isolate identified
169 as *A. tamarense* Tropical Asian ribotype does not belong to the species complex (John et
170 al., 2003b). A recent study that included gene sequence analysis on a worldwide basis
171 confirmed the clustering of ribotypes into five phylogenetically well-supported clades,
172 exclusively including either toxic or non-toxic strains (Lilly et al*.,* 2007). As this study
173 indicated that the geographic distinctions are no longer indicative of the range occupied
174 by members of each group, a group numbering scheme was introduced to replace
175 geographically referenced clade designations (Fig. 1).

176 Furthermore, morphological distinction of isolates of the different ribotypes shows
177 that phylogenetic clades are not reciprocally monophyletic. This lack of correlation of
178 morphological and molecular characters indicates that the taxonomic distinction of the
179 species *A. tamarense*, *A. catenella* and *A. fundyense* does not reflect the evolutionary

relationship within the species complex. Recent studies on reproductive traits of members of the complex support the notion that the evolutionary units as discerned by rDNA analyses are valid species according to a biological species concept (Brosnahan et al., 2010). In that study, isolates from different ribotypes were shown to be reproductively non-compatible by producing only non-viable zygotes (for the reproductive cycle of *Alexandrium,* see below).

Comparable findings have been obtained with other morphospecies. Isolates originally described as *A. angustitabulatum* and *A. lusitanicum* were found to be part of a species complex together with *A. minutum* (Franco et al*.,* 1995; Hansen et al*.,* 2003; Lilly et al., 2005). The analysis of globally distributed strains of the *A. minutum* species complex confirmed the identification of a distinct 'Pacific clade' clustering strains from New Zealand and a larger 'global clade' including both toxic and non-toxic strains, within which microsatellite markers revealed geographic structuring (McCauley et al., 2009). *Alexandrium andersonii* – the fourth member of the *A. minutum* group in the classification proposed by Balech (1995) – does not cluster close to the *A. minutum* clade, but rather in a clade with *A. ostenfeldii – A. peruvianum,* (e.g., Hansen et al., 2003; Touzet et al., 2008a) or branches off earlier (e.g., Penna et al., 2008; this study, Fig. 1), although overall support for any of these groupings is usually weak.

Alexandrium ostenfeldii and *A. peruvianum* are morphologically very similar, but can be separated based on their cell size, on the shape of the S.a. platelet, and the right anterior margin of the 1' plate (Balech, 1995). However, these characters showed considerable variation and overlapping in strains isolated from the Baltic Sea. Moreover, although the geographic coverage of the analyzed strains is still limited, there is evidence

for the presence of distinct genotypes, possibly cryptic species (Kremp et al., 2009). Similar findings have been obtained with *A. tamiyavanichi* and *A. cohorticula*. Again, detailed analysis of strains showed a broad range of characters that does not support their separation into distinct species (Lim et al., 2007; Menezes et al., 2010)

In the genus *Alexandrium,* as for many other protist taxa, the advent of molecular techniques challenged the classification of species based on morphological characters by showing that: i) a high level of genetic diversity is present within the same morphospecies, and ii) some characters for separation of closely related morphospecies show a broad range of variability and do not match molecular genetic clustering.

Morphological and genetic examination of strains obtained from different geographical locations, including the type locality of the different morphospecies, is required to formally re-define several species. Within *Alexandrium* it might be possible to identify 'species-complexes' that share some morphological characters. These complexes, however, will include a higher level of diversity that we now perceive as cryptic species (i.e., the *A. tamarense* ribotypes and clades within *A. minutum*) or distinct populations (e.g., the different population subclusters within *A. minutum* or *A. catenella*/Group IV as discriminated by microsatellite markers). Perhaps these are the 'units' to track if we are to understand the evolutionary history and dispersion patterns of these dinoflagellates.

One striking example that underlines the necessity of acknowledging molecular characters is the existence of strictly toxic and non-toxic ribotypes within the *A. tamarense* species complex (Scholin et al., 1994; Lilly et al., 2007). No consensus, however, has been reached on how to reconcile the molecular divergence of clades within

226 species complexes with respect to the taxonomic validity of described species and the
227 potential necessity to define new species on the basis of molecular or other hitherto
228 unrecognized characters. The development of a comprehensive species concept for
229 *Alexandrium* that acknowledges phylogenetic differences among evolutionary lineages
230 would certainly provide benefits for research, as distinctly evolved phylogenetic lineages
231 might differ substantially with respect to their ecological niches and bloom
232 characteristics.

233 **2.2 Species identification and discrimination**

234 Members of the genus *Alexandrium* are among the most difficult HAB taxa for
235 species identification because of the subtle morphological characteristics used for
236 classification, many of which are not easily resolved during monitoring or research
237 programs. Furthermore, as exemplified by the *A. tamarense* species complex, chain-
238 forming ability, thecal tabulation and cell shape (Balech, 1995) are considered by some to
239 be plesiomorphic features that are not reliable taxonomic markers (John et al., 2003b;
240 Leaw et al., 2005). Morphologically intermediate forms have been observed under
241 different environmental conditions both in culture and in the field (e.g., Anderson et al.,
242 1994), and toxic and non-toxic ribotypes of the same morphologically defined species
243 sometimes co-occur (e.g., Touzet et al., 2009; Brosnahan et al., 2010). Over the last few
244 decades, the introduction of a variety of molecular methods has made possible the
245 discovery of an incredible and unsuspected diversity within phytoplankton communities,
246 including within the genus *Alexandrium*.

247 A common approach taken with *Alexandrium* species involves the development of
248 species- or intra-specific molecular "probes" that can label cells of interest so they can be

249 detected visually, electronically, or chemically. Progress has been rapid and probes and
250 assays of multiple types are already available for many species and distinct ribotypes
251 (i.e., potential cryptic species). Although cell-surface antibodies have been used, the most
252 promising approach involves short pieces of synthetic DNA (probes or primers) that bind
253 to complementary portions of target molecules in the corresponding HAB species (Tables
254 2 and 3). These molecular targets, typically ribosomal RNA (rRNA), can be visualized
255 and/or quantified by a variety of techniques such as fluorescent in situ hybridization
256 (FISH); sandwich hybridization assays (SHA), and a variety of PCR-based assays
257 described below. These developments have reached the stage where the new molecular
258 counting methods are routinely employed in some research (e.g., Anderson et al., 2005b)
259 and monitoring programs.

260 **2.2.1 Amplification/sequencing-based methods**

261 rRNA genes have been widely used for identification and enumeration, as well as
262 for phylogenetic studies in *Alexandrium* (Table 2). Scholin and Anderson (1994, 1996)
263 were the first to use rRNA genes (small subunit or SSU, 18S rRNA; large subunit or
264 LSU, 28S rRNA) for *Alexandrium* identification and classification in a large-scale
265 restriction fragment-length polymorphism (RFLP) study that especially targeted species-
266 and group-specific sequence differences in these genes.

267 Among the ribosomal genes, the D1/D2 region of LSU rDNA has also revealed
268 evolutionary relationships and species boundaries within the *A. minutum* group (Lilly,
269 2003), and thus it has been the basis of numerous identification and biogeographical
270 studies worldwide (Lilly et al., 2002; MacKenzie et al., 2004; Ruiz Sebastian et al., 2005;
271 Menezes et al., 2010). Similarly, multiplex PCR assays have been developed, based upon

primers designed from the D1/D2 and ITS regions, for the simultaneous detection and quantification of *Alexandrium* species coexisting in French and Japanese waters (Guillou et al., 2002; Genovesi et al., 2011; Nagai, 2011) and *Alexandrium* cysts in bottom sediments (Erdner et al., 2010).

The rRNA gene has also been used for quantification of *Alexandrium* cells, such as those of *A. minutum,* by addressing the 5.8S rDNA from both preserved environmental samples and cultures (Galluzzi et al., 2004). However, it was recently shown that rRNA gene copy number significantly varies even among *Alexandrium* species, and at least within *A. taylori* also according to growth phase (Galluzzi et al., 2010; Brosnahan et al., 2010). This is a critical consideration when applying quantitative PCR-based techniques for cell enumeration.

Mitochondrial markers have recently emerged as a powerful alternative for species discovery and identification. Under the name of DNA barcoding, these markers, such as the cytochrome c oxidase subunit 1, are used to discriminate unidentified taxa and to assign them to species. However, when applied for the investigation of dinoflagellate diversity, DNA barcoding with mitochondrial markers failed to resolve strains belonging to the genus *Alexandrium* (e.g., Lin et al., 2009; Stern et al., 2010).

2.2.2 Hybridization-based methods

Hybridization protocols based upon taxon-specific molecular probes targeting rDNA regions have also been developed to enable the rapid detection of individual *Alexandrium* species using FISH, SHA, or PCR-based assays (Table 3). This work has been especially productive for the *A. tamarense* species complex, as well as for *A.*

294 *minutum* and *A. ostenfeldii* (e.g., Penna and Magnani, 1999; Metfies et al., 2005; John et
295 al., 2005; Diercks et al., 2008; Gescher et al., 2008; Touzet et al., 2010; Erdner et al.,
296 2010).

297 DNA microarrays (or "chips") allow the simultaneous analysis of several target
298 genes or taxa in a single experiment, and as such represent a useful tool for studying
299 complex phytoplankton communities. The ALEX CHIP (Gescher et al., 2008) was the
300 first prototype developed for the detection of several *Alexandrium* species. The newly
301 developed biosensor ALGADEC (Diercks-Horn et al., 2011) enabled the detection of *A.*
302 *minutum* in a semi-automated fashion. In this regard, it appears as a promising device for
303 the study of HABs. The possibility of combining multiple probes targeting multiple
304 species makes this sensor, and related multiplex instruments (e.g., Scholin et al., 2009),
305 an effective approach for detection and quantification of toxic algae in the field.

306 **2.3 Biogeography and evolution**

307 Members of the genus *Alexandrium* are widespread globally, with species present
308 in coastal, shelf and slope waters of subarctic, temperate and tropical regions of the
309 Northern and Southern Hemispheres (Taylor et al., 1995; Lilly et al., 2007). The diversity
310 of *Alexandrium* appears to be higher in the Mediterranean Sea than elsewhere, but this
311 may reflect the level of taxonomic scrutiny more than an actual distribution. For
312 illustration, twelve distinct species (including three ribotypes of the *A. tamarense* species
313 complex) have been identified so far from this regional sea (Penna et al., 2008; Fig. 2).
314 The *A. tamarense* species complex appears to be the most widely dispersed and occurs in
315 many locations worldwide, covering all ocean basins and many regional seas (Lilly et al.,

316 2007). On the other hand, members of this species complex seem to be largely absent
317 from the equatorial tropics.

318 Whereas many biogeographical studies of *Alexandrium* are based upon
319 examination of vegetative cells, the hypnozygotes or cysts are highly resistant to decay
320 and thus facilitate studies of the distribution of some species in modern sediments and
321 their linkages with environmental conditions. Cysts of *A. tamarense* have been found
322 within a surface water temperature range of -0.6 to 26.8°C with the highest relative
323 abundances in regions between 5 and 15°C. Members of this species complex can be
324 regarded as characteristic of temperate/subtropical regions in brackish to fully marine and
325 oligotrophic to eutrophic environments (Marret and Zonneveld, 2003).

326 Although many *Alexandrium* species are known to be widely distributed across
327 several continental coastal and shelf waters, comprehensive distributional data for many
328 regions are still scarce. Hence, the underlying biogeographic constraints and natural
329 distributional patterns remain largely obscure. Nevertheless, for a few species, such as
330 those from the *A. tamarense* species complex, the observed distributional patterns were
331 seemingly dense enough to formulate an evolutionary model based on vicariance and
332 allopatric speciation to explain the present day distribution as a consequence of plate
333 tectonics, long-term climate variation and related alterations in paleoceanographic
334 conditions (Scholin et al., 1994; John et al., 2003b). In other *Alexandrium* species, the
335 formation of genetic population structure and eventually the divergence of evolutionary
336 lineages are most likely driven by the same factors. An understanding of differentiated
337 evolutionary lineages with distinct biogeographies in other species or species complexes,
338 such as *A. minutum* (Lilly et al., 2005; McCauley et al., 2009), *A. ostenfeldii* (Kremp et

al., 2009), *A. tamiyavanichi* (Menezes et al., 2010), is already emerging. As more detailed studies on these taxa are carried out, common patterns may become prominent for the evolutionary forces shaping *Alexandrium* species and populations.

Over the last century, these natural processes have been augmented by human activities such as ballast water discharge (e.g., Bolch and de Salas, 2007) or shellfish stock transfers. Some argue that the dramatic increase of recorded HAB events and changes in their intensity over the last decades are at least partially a consequence of human-mediated range extensions of HAB species, including those of *Alexandrium* (Hallegraeff, 1993; Masó and Garcés, 2006). One example is seen in the Mediterranean Sea, which harbors a large number of reportedly invasive toxic and non-toxic *Alexandrium* species. *Alexandrium catenella* was first reported in the Balearic Islands and Catalonia in 1983 (Margalef and Estrada, 1987), and then appears to have spread in the Western Mediterranean region along the French, Spanish, Italian, Greek and Maghrebian coasts (Abadie et al., 1999; Vila et al., 2001; Lugliè et al. 2003; Frehi et al., 2007; Turki et al., 2007).

The emergence of molecular techniques that enable high-resolution genetic characterization of a population will lead to a reexamination of some of these invasion reports. In some cases, species considered as exotic may turn out to be part of a "hidden flora", and their emergence may then be attributed to climate change or to other processes that alter the environment in a way that favors their detection (Smayda, 2007). To this end, polymorphic genetic markers such as DNA microsatellites have been developed for some *Alexandrium* species (e.g., *A. tamarense* North American clade/Group I (Nagai et al., 2004; Alpermann et al., 2006), *A. minutum* (Nagai et al., 2006a), *A. catenella*

Temperate Asian clade/Group IV (Nagai et al., 2006b). An example of the application of these versatile molecular tools is in understanding the sudden appearance of *A. catenella* in Thau Lagoon in the Mediterranean after decades of non-detection during monitoring programs. On the basis of rRNA sequencing, this was argued to be a result of human-assisted introduction (Lilly et al., 2002). However, when Masseret et al. (2009) examined these same strains using hypervariable microsatellite markers, relationships emerged that were not apparent from rRNA studies on the same group. Mediterranean populations were shown to be a distinct lineage and therefore other origins must now be explored.

Detailed analyses of past range extensions and ongoing population differentiation require concerted research efforts with regard to population sampling and method development (e.g., of genetic markers for single-cell genotyping). One such successful effort has been the transregional analysis of population genetic structure of the *A. tamarense* Group I clade from Japan and Korea (Nagai et al., 2007). Here the degree of genetic differentiation of populations was strongly and positively correlated with geographic distance of sampled populations. However, the observed genetic patterns also allowed identification of some geographically defined populations with deviations from the general model that were most plausibly explained by human mediated interference, e.g., by transfer of *A. tamarense* cells with shellfish stocks.

A recent study that combined genetic models and indirect connectivity, as estimated by oceanographic modeling, showed the existence of a genetic population substructure for *A. minutum* in the Mediterranean Sea (Casabianca et al., 2011). The observed regional genetic structure (i.e., existence of four distinct genotype clusters in their majority formed by isolates from the Adriatic, Ionian, Tyrrhenian or Balearic-Tyrrhenian Sea) was

385 explained by basin-scale transportation patterns through successive generations of
386 vegetative microalgal cells. In contrast to earlier expectations of broad genetic uniformity
387 in planktonic marine microbes, which were based on assumptions of high dispersal
388 capabilities and large population sizes, such strong intraspecific regional genetic patterns
389 might be observed for the majority of *Alexandrium* species and other microorganisms.
390 This is especially true when complex ecological requirements may pose barriers to
391 dispersal during different stages of their life cycles.

392 One fascinating aspect of *Alexandrium* biogeography is the distribution of toxic and
393 non-toxic strains of the same species, or of closely related species. Generally, the
394 distributions do not overlap, as is the case for *A. minutum* in Ireland, where toxic forms
395 are found in the south, and non-toxic strains in the west (Touzet et al., 2008a). Two
396 known exceptions are the Shetland Islands in Scotland (Touzet et al., 2010), and Belfast
397 Lough in Northern Ireland (Brosnahan et al., 2010). Toxic and non-toxic species within
398 the *A. tamarense* complex have been documented in both locations. A possible
399 explanation for this distinct range separation of toxic and non-toxic strains or species was
400 recently demonstrated by Brosnahan et al. (2010) who mated Group I and Group III
401 strains of *A. tamarense* (toxic and non-toxic, respectively), forming true resting cysts that
402 germinated, but the germling cells could not survive. This reproductive barrier argues that
403 Group 1 and Group III ribotypes are different biological species and also suggests that
404 biogeographic patterns might be shaped by limited sexual compatibility. Invasions by one
405 type into the range of another may not be successful unless it arrives in overwhelming
406 numbers, because hybridizations are lethal.
407

3 Life Histories

3.1 Life cycle generalities and unique aspects for different species

The life cycle of *Alexandrium* species investigated thus far – as that of most protists – includes different stages that have distinct morphology, physiology and function. Although sharing the same genetic material, the cells of different life cycle stages within a population have important and different functions, but the environmental and/or internal signals that induce transition between those stages are still largely unknown (von Dassow and Montresor, 2011). The reconstruction of the general life cycle pattern, i.e. of the different life stages, can be achieved only with laboratory investigations where cultures are studied under different experimental conditions. Nevertheless, *in situ* studies provide the necessary validation of the experimental approach and are in turn source of new questions for experimental work.

The general scheme of the life cycle of *Alexandrium* species (Fig. 3) can be summarized as follows. There are, however, various aspects (indicated in parentheses below) that may vary from species to species and even among genetically distinct strains of the same species:

- haploid motile stages (cell division modality; chain formation)
- asexual cysts, i.e. pellicle cysts
- haploid gametes (homothallic, heterothallic or complex mating system)
- diploid zygote (fate of the zygote: remains motile, transforms into a long-lived resting cyst, or into a short-term cyst that germinates rapidly)
- diploid non- motile cyst (length of the dormancy period; factors that regulate germination)

3.1.1 The vegetative phase

Alexandrium species – as almost all dinoflagellates – are haploid during their vegetative phase; the diploid stages are the planozygote produced following gamete conjugation (Figueroa et al., 2007) and the sexual cyst or hypnozygote. Vegetative cell division usually occurs through desmoschisis (Figueroa et al., 2007), i.e. each daughter cell maintains half the thecal plates of the mother cells, and couplets of recently divided cells are often recorded in actively growing cultures. A phased cell cycle, with maxima of dividing cells recorded shortly before the end of the dark phase, has been reported for *A. minutum* (Probert et al., 2002). However, the formation of non-motile division cysts has been reported for three species of the subgenus *Gessnerium*: *A. pseudogonyaulax, A. taylorii* and *A. hiranoi* (Kita et al., 1985; Montresor, 1995; Garcés et al.; 1998). In *A. pseudogonyaulax,* cells cast off thecal plates and flagella and two (or at times four) flagellated daughter cells emerge from the division cyst (Montresor, 1995). In natural populations of *A. hiranoi* (reported as *A. pseudogonyaulax* in Kita et al., 1985), division cysts are produced at the beginning of the dark period. They settle on the sediments and release two flagellated daughter cells after the initiation of the light phase. In *A. taylorii*, both vegetative division modalities have been reported (Garcés et al., 1998; Giacobbe and Yang, 1999) namely the formation of division cysts, within which 2, 4 or 8 cells were produced, and division through desmoschisis. In the natural environment, the formation of division cysts shows some evidence of a daily rhythm, being preferentially restricted to the dark phase (Garcés et al., 1998).

Chain formation is a definable species characteristic that also represents an example of life stage transition within the vegetative phase; the capability to form long

chains is reported for several species such as *A. catenella*, *A. affine, A. fraterculus, A. cohorticula,* and *A. tamiyavanichi*. Chain formation in *A. catenella* may be stimulated by turbulence (Sullivan et al., 2003), and chain length may decrease in culture, thus suggesting that this feature represents an adaptation to high turbulence upwelling systems. However, this interpretation does not apply to *A. catenella* isolated from Thau Lagoon (Northern Mediterranean), as strains have a high sensitivity to agitation in culture (Collos et al., 2004). Chains of cells have a faster swimming velocity than single cells (Fraga et al., 1989) and might thus migrate diurnally between the deep nutrient-rich layer and the surface. The capability to switch between single cells and chains might also represent a strategy to reduce grazing.

Another stage transition within the vegetative phase is represented by the formation of pellicle cysts, which are non-motile cells surrounded by a thin wall (Anderson and Wall, 1978; pellicle cyst terminology reviewed in Bravo et al. (2010)). Pellicle cysts can be formed as a reaction to environmental stress conditions such as turbulence, the presence of parasites, or passage through the gut of grazers. Pellicle cysts have no mandatory maturation period and can revert to the vegetative motile stage once stress conditions are over. The capability to rapidly turn into a pellicle cyst might represent an effective defense strategy against parasite attacks. In fact, when *A. ostenfeldii* was exposed to the parasitic flagellate *Parvilucifera infectans* or to waterborne cues produced by them, a large fraction of the population became temporary cysts, which were more resistant to parasite infection (Toth et al., 2004).

3.1.2 The sexual phase

Gametes of *Alexandrium* species are either undifferentiated from vegetative cells or are smaller in size. The mechanisms leading to the differentiation of gametes, as well as the modalities of the recognition system between gametes are still unknown. In induction of the sexual phase, conjugation starts after cells pair, facing their ventral side. The appearance of conjugating gametes and formation of larger and biflagellate planozygotes is generally obtained by transferring vegetative cells into diluted N- or P-deprived culture medium (e.g., Anderson and Lindquist, 1985). However, the difficulty of distinguishing gametes in natural populations limits the possibility to link specific nutritional factors with the onset of the sexual phase. In *A. hiranoi,* formation of smaller division cysts producing four smaller motile cells has been interpreted as the process leading to the formation of gametes; these smaller cells fuse and produce a biflagellate swimming zygote or planozygote (Kita et al., 1993). The inhibitory effect of concavalin A and tunicamycin on the conjugation process in *A. catenella* has been interpreted as evidence for agglutinin-like compounds involved in gamete-gamete recognition (Sawayama et al., 1993).

In the last decade, evidence has been provided for a number of cyst-forming dinoflagellate species, including some *Alexandrium* (*A. minutum, A. tamutum* (Figueroa et al., 2007)*, A. taylorii* (Figueroa et al., 2006), *A. catenella* (Figueroa et al., 2005), *A. peruvianum* (Figueroa et al., 2008a)) that the transition between planozygote and resting cyst is not an obligate one. Furthermore, the planozygote can indeed undergo multiple alternate transitions, depending on environmental conditions. In *A. taylorii*, the planozygote can either undergo cell division to produce two vegetative cells, or transform

498 into a short-term pellicle cyst, or into a long-term resting cyst (Figueroa et al., 2006).
499 When pairing gametes were isolated into different culture media, direct division prevailed
500 in nutrient replete media, whereas the formation of pellicle cysts mainly occurred in P-
501 depleted medium or in diluted medium, and the formation of thick-walled resting cysts
502 was only observed in N-depleted media. However, the response of planozygotes to
503 different nutrient conditions does not follow a consistent pattern amongst species. In fact,
504 encystment of *A. catenella* planozygotes was high both in N-depleted medium and in
505 nutrient replete conditions (Figueroa et al., 2005). The high production of pellicle cysts
506 observed in P-depleted medium for *A. taylorii* was confirmed, and pellicle cysts were
507 able to germinate into a motile vegetative cell within a few days. A similar life cycle
508 pattern in which the planozygote either divided – when transferred into nutrient-replete
509 medium – or transformed into a short-term pellicle cyst when incubated in N- or P-
510 depleted medium was described for *A. peruvianum* (Figueroa et al., 2008a). The
511 formation of sexual resting cysts in this species was observed in culture when mixing
512 strains of opposite mating type, but never observed when individual planozygotes were
513 isolated into different media. This raises the possibility that other factors, such as cell
514 concentration (Uchida, 2001), might play a role in determining the fate of planozygotes.

515 *Mating system*

516 The mating system can be assessed by detecting the formation of zygotes in
517 clonal strains, or in pair-wise crosses of clonal strains. In fact, assuming that cysts
518 represent the diploid stage deriving from the fusion of two gametes, the culture resulting
519 from the germination of a cyst contains a mixture of the two parental types. Moreover,
520 evidence for sexual compatibility should be provided by the observation of planozygotes

and not only by resting cysts, due to the fact that the two processes might be uncoupled, i.e. planozygotes can be produced but they do not necessarily transform into cysts. Homothallic, heterothallic, and more complex mating systems have been reported within the genus *Alexandrium*. The first mating studies carried out on *A. catenella* (Yoshimatsu, 1981, 1984) demonstrated a heterothallic mating system, and that the chain of cells produced from the germination of a sexual cyst included two different mating types, i.e. cells in the posterior and anterior half of the chain were different types. In contrast, experiments on monoclonal strains suggested a homothallic system for *A. affine* (Band-Schmidt et al., 2003). The mating system of *A. tamarense* (as *A. excavatum*) and the reproductive efficiency was investigated by crossing multiple clonal strains and monitoring the presence of fusing gametes, cyst formation and subsequent germination success (Destombe and Cembella, 1990). Both auto-compatible (putatively homothallic) and heterothallic strains were determined, and one strain was capable of crossing with all the others, suggesting that this species has a complex mating system. This system involves a spectrum of mating compatibility rather than two defined mating types, a finding confirmed by Brosnahan et al. (2010).

Cyst formation, maturation, and germination

The planozygote formed from gamete fusion can follow different routes, one of which is the formation of hypnozygotic resting cysts, when there is a temporary suspension of germination due to both exogenous and endogenous factors. The length of the maturation period during which germination of newly formed cysts is not possible even under favorable conditions and the factors that induce and modulate encystment and excystment are important in population dynamics. For *Alexandrium* species studied in the

544 laboratory, encystment has been induced by inoculating strains into culture medium with
545 reduced concentration of N- or P- nutrients or into diluted media (e.g. Anderson et al.,
546 1984; Figueroa et al., 2005). Besides depleted nutrients, other factors might influence
547 encystment success (see Olli et al. (2004)) for a discussion of methods and terminology
548 to quantify encystment). Cyst production may vary with temperature (e.g., Anderson et
549 al., 1984) and specific bacteria can play a role in inducing or inhibiting encystment in *A.*
550 *tamarense* (e.g., Adachi et al., 1999).

551 Estimates of the length of the maturation period range widely, from 2 months for
552 the tropical *A. affine* (Band-Schmidt et al., 2003), 28-55 days for Tasmanian populations
553 of *A. catenella* (Hallegraeff et al., 1998), 1-3 months for *A. peruvianum* (Figueroa et al.,
554 2008a), and 12 months for *A. tamarense* from the St. Lawrence estuary (Castell Perez et
555 al., 1998). When maturation is complete, cysts can germinate if permissive environmental
556 conditions are met. Storage of cysts in the dark and at low temperature synchronized the
557 germination of *A. pseudogonyaulax* cysts upon their re-exposure to the light (Montresor
558 and Marino, 1996). The composition of the encystment medium can also modulate the
559 length of maturation period in *A. catenella*; cysts produced in a diluted medium had a
560 longer maturation period than those produced in N- or P-depleted conditions (Figueroa et
561 al., 2005). Furthermore, maturation took longer when cysts were incubated in full
562 strength medium versus in seawater. Above all, a considerable difference in maximum
563 germination frequency and in germling viability has been detected amongst experiments
564 carried out with different parental strains, further complicating the delineation of the
565 factors that regulate life cycle transitions. These results call for comparative studies

566 carried out using standardized experimental protocols with different strains for each
567 species, and/or with populations from different geographic areas.

568 Information on excystment patterns and rates has been obtained from natural cyst
569 assemblages stored under conditions comparable to those recorded in the field, and re-
570 suspended in the light (and at times also in the dark) over a range of temperatures. The
571 advantage of this approach is that cysts are produced under natural conditions and
572 represent the integrated response to environmental factors. Cysts of *A. tamarense*
573 collected in the Cape Cod area had a temperature window for germination between 5 and
574 21 °C (Anderson and Rengefors, 2006). Natural cyst assemblages of the same species
575 collected from Japanese coastal sediments and incubated at conditions matching those
576 recorded in the field showed a clear seasonal pattern of germination, related to low
577 temperature conditions (10-15 °C) in the bottom sediments (Itakura and Yamaguchi,
578 2001). A broad temperature window for germination (2-16 °C) was described for *A.*
579 *tamarense* cysts collected in the cold St. Lawrence estuary (Castell Perez et al., 1998).
580 Excystment was not triggered by exposure to the light or by temperature shifts. The
581 germination of cysts in natural sediments showed a marked seasonality with higher
582 values (>50%) from August to October. The results argued for either a temperature-
583 controlled cyst maturation period, i.e., in colder waters the maturation period is longer, or
584 an endogenous annual clock that controls the timing of germination. Evidence for the
585 second mechanism had been provided for *A. tamarense* populations collected from the
586 Gulf of Maine, where a clear seasonal pattern of cyst germination was detected under
587 constant conditions and for multiple successive annual cycles (Anderson and Keafer,
588 1987).

589 Yet another variation of this mechanism was recently reported by Ni Rathaille and
590 Raine (in press), who could not detect an endogenous annual clock in laboratory-stored *A.*
591 *minutum* and *A. tamarense* cysts from Cork Harbor, Ireland. Instead they found
592 seasonality in germination in cysts collected repeatedly from natural sediments. This
593 suggests a type of secondary dormancy (found in higher plants), whereby cyst
594 germination is seasonal, but the patterns of that regulation are determined by the external
595 environment.

596 **3.2 Role of cysts in population dynamics**

597 A common assumption is that cyst "seedbeds" provide the inoculum for blooms of
598 cyst-forming *Alexandrium* species. The concept of a discrete seedbed may not be
599 appropriate in some locations, however, due to the widespread, dispersed distribution of
600 some cysts and the likelihood that germination will occur over a large area. Nevertheless,
601 there is evidence for localized cyst accumulations, both in estuarine systems and in
602 deeper coastal waters, so perhaps these features are more common than previously
603 expected. For example, cyst mapping within the Nauset Marsh System on Cape Cod
604 revealed three highly localized seedbeds at the extreme ends of the complex network of
605 channels and salt ponds that comprise that system (Crespo et al., in press.). Not only are
606 the cysts of *A. fundyense* found predominantly in three kettle holes or salt ponds, with
607 virtually no cysts in between, but detailed field surveys during bloom season documented
608 the tight link between these cyst seedbeds and the areas of bloom initiation and retention
609 within the system. A similar linkage between cyst accumulations in lagoons, harbors, or
610 other such sites is found in the Mediterranean, and is responsible for localized blooms of
611 *A. catenella* in Thau Lagoon (Genovesi et al., 2009) and Tarragona Harbor (Bravo et al.,

612 2008). Examples of cyst seedbeds in deeper coastal waters are less common, perhaps due
613 to the expense and difficulty of large-scale mapping, but some large studies have been
614 conducted, revealing accumulations stretching hundreds of km along the shore, and 50
615 km or more offshore, such as those for *A. fundyense* in the Gulf of Maine (e.g., Anderson
616 et al., 2005c).

617 In temperate regions, *Alexandrium* cysts remain quiescent during the winter months
618 i.e., the cysts are mature and capable of germination, but are prevented from doing so by
619 cold temperatures (Anderson, 1998; Anderson and Rengefors, 2006). As discussed above,
620 a remarkable second level of germination control has been demonstrated for *A. fundyense*
621 cysts and for which an internal, annual clock restricts germination to certain times of the
622 year (Anderson and Keafer, 1987; Matrai et al., 2005). This endogenous annual clock
623 drives the seasonality of *A. fundyense* blooms in deeper, coastal waters where
624 environmental cues in bottom waters are weak.

625 Anoxia is yet another factor that regulates cyst germination, because cysts can
626 germinate only in the presence of oxygen (Anderson et al., 1987). In bottom sediments,
627 this tends to comprise only those cysts found at the very surface – perhaps the top few
628 millimeters. The number of cysts that contribute to the bloom initiation process is
629 therefore generally small relative to the total number in the sediments. This is in part
630 because more cysts are often buried below the sediment surface than are present in the
631 top, oxygenated layer (Anderson et al., 1982).

632 The size of the cyst germination inoculum from this surface layer may be small.
633 For example, evidence is now emerging from germination flux experiments in Japanese
634 embayments (Ishikawa et al., 2007) or in temperate salt ponds on Cape Cod (E. Vahtera,

635 unpub. data) that germination rates are a fraction of a percent per day – meaning that 20%
636 or less of the cysts in the top few millimeters of surface sediments might germinate in a
637 6-8 week season with a germination flux rate of only ~0.4% day^{-1}. With typical *A.*
638 *fundyense* cyst concentrations in surface sediments in Cape Cod salt ponds (Crespo et al.,
639 in press), a week of germination would lead to an inoculum cell concentration of ~70 -
640 100 cells L^{-1} at bloom initiation, roughly equivalent to what has been observed in the
641 early stages of such blooms (Anderson et al., 1983; Crespo et al., in press). In subsequent
642 weeks, the germination flux would be similar, but those cells would be greatly
643 outnumbered by dividing cells in the water column. With an estimated inoculum of this
644 size, the magnitude of the resulting bloom population appears to be regulated by factors
645 affecting cell growth and retention, and not by the abundance of cysts in bottom
646 sediments.

647 As is the case with localized salt ponds and embayments discussed above,
648 examples of discrete cyst seedbeds that lead to large-scale regional blooms do exist.
649 Quantitative cyst maps in deeper, open coastal waters are available for *A. tamarense* and
650 *A. fundyense* (e.g., Anderson et al. 2005c), *A. catenella* (e.g., Yamaguchi et al*.,* 1995), *A.*
651 *minutum* (Erard-LeDenn et al*.,* 1993) and *A. ostenfeldii* (MacKenzie et al*.,* 1996).
652 Cembella et al. (1988) argue that *A. tamarense* cysts along the northern shore of the St.
653 Lawrence estuary initiate the toxic blooms which cause PSP on the south shore and
654 further downstream in the estuary. On the northeast coast of Britain, *A. tamarense* cyst
655 accumulations in the Firth of Forth have been linked to toxic blooms in the adjacent
656 coastal waters to the north (Lewis et al., 1995). Evidence for the existence of a regional
657 seedbed is also found in studies in the Gulf of Maine where a strong correlation between

658 the abundance of *A. fundyense* cysts and the size of subsequent blooms (expressed as the
659 extent of PSP toxicity closures along the coast) has been documented (McGillicuddy et
660 al., in press).

661 **3.3 Role of cysts in maintaining population genetic structure and functional**
662 **diversity**

663 Cysts are long-lived and can be expected to contribute not only to initiation of
664 planktonic populations in the next planktonic growth phase, but as well – although
665 presumably to a lesser extent – to that in consecutive years. Patterns of excystment and
666 subsequent survival and growth are therefore suggested to have considerable influence on
667 the genetic structure of *Alexandrium* populations. According to a conceptual model,
668 derived from microsatellite- and AFLP-based population genetic analyses, cyst seedbeds
669 of *Alexandrium* harbor a similar population genetic structure and diversity to that found
670 in planktonic populations (Alpermann et al., 2009). Interannual differentiation of
671 planktonic populations as the result of clonal selection and shifts in genotype frequencies
672 due to variations in selective constraints of the environmental regimes is the most likely
673 explanation for observed population genetic substructures. Within a single year,
674 environmental selection for differential growth and encystment can similarly act to
675 establish and reinforce population structure. For example, an *A. fundyense* (Group I)
676 bloom in the northeastern U.S. was shown to contain at least two genetically distinct sub-
677 populations, comprising either early-bloom or late-bloom samples, whose succession is
678 presumably influenced by environmental conditions (Erdner et al., 2011). These temporal
679 differences in population composition are reinforced during the mating and encystment
680 process, as the most probable matings will occur between genotypes from the same sub-

681 population. The resulting cysts will be deposited at different times during the bloom but
682 maintain the distinctive genetic signatures of their sub-populations, thereby maintaining
683 the diversity of the overall regional cyst pool. The phenotypic adaptations of the progeny
684 resulting from the germination of the resting cysts, may be the result of the exogenous
685 environmental factors and the parental origin, as was first demonstrated by Figueroa et al.
686 (2005) with *A. catenella* monoclonal cultures. With their diverse composition of
687 descendants derived from successful growth of planktonic vegetative cells from different
688 years, benthic cyst seedbeds constitute a genetic repository and may contribute
689 substantially to the persistence of resident populations of *Alexandrium* by retaining a high
690 degree of functional genetic diversity.

691 **4 Physiology and Nutrition**

692 The traditional diatom bloom model cannot adequately describe *Alexandrium*
693 blooms; as mentioned by Heisler et al. (2008), we need to "move away from simplistic
694 inorganic nutrient-dose-yield models". Although *Alexandrium* is an opportunistic genus
695 relative to nutrition, simple relationships with classical nutrients should not be expected.
696 *Alexandrium* has the ability to grow in both nutrient-rich (Townsend et al., 2005;
697 Spatharis et al., 2007) in relatively pristine waters (Anderson et al., 2002), but also in
698 waters where nutrient abatement has been carried out (e.g., Collos et al., 2009). It is
699 difficult therefore to generalize about the nutrient-niche of *Alexandrium*, and the nutrient-
700 dependent mechanisms that select for individual genera and among species that will
701 bloom.

4.1 Carbon

Alexandrium species take up inorganic C and produce oxygen like other autotrophs, but, as for other dinoflagellates, respiration (R) appears to be higher than in other phytoplankton classes, both relative to gross photosynthesis (PS) (Falkowski and Owens, 1978) and growth rate (Langdon, 1987). This is thought to be due to high energy requirements for maintenance of their large genome, with motility costs assumed to be negligible (Raven and Richardson, 1984). The compensation irradiance (when PS=R) for *Alexandrium tamarense* (= *Gonyaulax tamarensis*) was also found to be higher than for representatives of other phytoplankton classes (Falkowski and Owens, 1978). This tends to indicate that *Alexandrium* can be adapted to high irradiances (Smayda, 2008), although evidence to the contrary also exists (Chang and McClean, 1997). No photoinhibition of growth could be shown up to 800 µmol photons m^{-2} s^{-1} for a Chilean strain of *A. catenella* (Carignan et al., 2002), but high sensitivity to UVB radiation was demonstrated.

Inorganic C losses through respiration are probably important, but there is apparently very little excretion of organic C by *Alexandrium* (Chen and Wangersky, 1996; Flynn et al., 2008). Inorganic C fixation was found to be influenced by N uptake, either decreasing (Collos et al., 2004, 2007), or increasing (Leong et al., 2010) as N uptake increased, depending on the cell nutritional state. Uncoupling of C and N metabolism is also exemplified in cultures with large (2 to 4-fold depending on species and/or strains) increases in C/N ratios following N exhaustion over time scales of 10 to 17 days (Flynn et al., 1996). Diel changes in C/N cellular ratios also occur in *A. tamarense* (MacIntyre et al., 1997) and *A. catenella* (Collos et al., 2006). In the former,

724 the amplitude of such variations was higher under N-deficiency (11-18 molC/molN) than
725 under N-sufficiency (7-10 molC/molN).

726 **4.2 Nitrogen**

727 *Alexandrium* growth rates on nitrate, ammonium and urea have been compared in
728 many laboratory culture studies (e.g., Levasseur et al., 1995; Matsuda et al., 1999;
729 Hamasaki et al., 2001; Dyhrman and Anderson, 2003). Generally, growth rates on
730 ammonium are higher than on nitrate, but the differences are not always significant,
731 except for one *A. catenella* strain (Dyhrman and Anderson, 2003). Urea is taken up by
732 *Alexandrium* and typically supports growth in both laboratory cultures and in the field
733 (Collos et al., 2007). Growth on urea may be lower than on either nitrate or ammonium,
734 but again, the differences are not substantial, except for a strain of *A. catenella* (Matsuda
735 et al., 1999) and one of *A. fundyense* (Levasseur et al., 1995), for which no growth was
736 reported with urea as the sole N-source. John and Flynn (1999) reported that amino-N
737 from amino acids cannot support significant growth of *A. fundyense*. The differences in
738 N-dependent growth observed among strains must be tempered with the caveat that
739 background N concentrations and sources were not always well controlled.

740 Early studies on *A. tamarense* showed that soil extract could increase growth
741 relative to that on purely inorganic medium (Prakash, 1967). More detailed work
742 confirmed the role of humic substances in enhancing growth in various media (Prakash
743 and Rashid, 1968; Gagnon et al., 2005). In the latter study, humic additions significantly
744 enhanced growth rates of *A. tamarense* relative to controls. Concentrations of these
745 humic substances remained constant throughout exponential growth phase, suggesting
746 that they were acting mainly as growth promoters. Carlsson et al. (1998) reported an

747 increase in *A. catenella* growth rate on nitrate-based medium when humic substances of
748 terrestrial origin were added. Doblin et al. (2001) showed that humic substances in
749 equimolar concentrations could replace nitrate as an N source and support similar growth
750 rates of the same species.

751 Riverine dissolved organic nitrogen (DON; >1 kDa) did not yield significant
752 differences between various ratios of NO_3/DON on growth of *A. tamarense* in f/2
753 medium, although chlorophyll content decreased as riverine DON increased (Stolte et al.,
754 2002). In contrast, Fagerberg et al. (2009) reported that *A. minutum* could benefit from
755 riverine high molecular weight (10-100 kDa) DON. Similarly, DON from marine diatom
756 blooms significantly increased (by 34%) the growth rate of *A. catenella* in cultures
757 (Loureiro et al., 2009) relative to growth on nitrate only. Ammonium was not responsible
758 for the increased growth, implying that DON was used directly.

759 Nitrogen uptake kinetics of *Alexandrium* species are not very different from those
760 of other phytoplankton (Kudela et al., 2010), with the possible exception of linear
761 kinetics, (i.e. no substrate saturation) for urea uptake (Jauzein et al., 2008a), N-loss
762 exemplified by release of nitrite during nitrate assimilation (Flynn and Flynn, 1998) and
763 release of ammonium during urea assimilation (Jauzein et al., 2008a). Multiphasic
764 kinetics allow *Alexandrium* species to exploit patches of elevated nutrient concentrations,
765 but they are also competitive at scavenging low N levels (e.g., Collos et al., 2007). In
766 some cases, substrate inhibition of uptake occurs for ammonium at concentrations of 100
767 μM (Leong et al., 2010).

768 There are also large intra-specific differences in uptake and assimilation kinetics
769 (Collos et al., 2006; Jauzein et al., 2008a). Furthermore, for a given strain, changes in

770 kinetic parameters, such as the half-saturation constant (K_s) and maximum uptake rate
771 (V_{max}) occur over the course of a day for both ammonium and urea, in relation with the
772 daily irradiance change (Jauzein et al., 2008a). In natural populations of *A. catenella*, K_s
773 for ammonium can change by an order of magnitude over a time scale of a few days
774 (Collos et al., 2007).

775 Dark uptake has been observed in *Alexandrium* but mostly for ammonium and urea,
776 with very little nitrate uptake occurring in the dark (MacIsaac et al., 1979), or most nitrate
777 being released as nitrite (Flynn and Flynn, 1998). The dark/light uptake ratios were
778 related to the oxidation state of the N-source (Leong et al., 2010).

779 The nitrate uptake system of *A. catenella* and *A. minutum* were shown to be very
780 sensitive to inhibition by ammonium (Collos et al., 2004; Maguer et al., 2007).
781 Ammonium was also found to inhibit the urea uptake system of *A. catenella*, but this
782 phenomenon seemed to be strongly strain-dependent. Whereas strains from Thau lagoon
783 on the French Mediterranean coast were very sensitive, strains from the Spanish
784 Mediterranean coast were much less so, indicating a possible geographical difference
785 linked to different nutrient regimes (Jauzein et al., 2008b).

786 *Alexandrium* cells accumulate ammonium internally but there are large inter-
787 specific (Thoresen et al., 1982; Flynn and Flynn, 1998) as well as intra-specific (Collos et
788 al., 2006) differences. In some cases, internal ammonium can represent up to 30% of the
789 total cell N of *A. catenella* strain TL01 (Collos et al., 2006), a high value for
790 phytoplankton but average for dinoflagellates; this was related to high uptake rates.
791 Compared to other dinoflagellates, the N physiology of *Alexandrium* species is

792 characterized by abnormally high internal levels of glutamine and arginine, as possible
793 precursors of PSP toxins (e.g., Anderson et al., 1990).

794 **4.3 Phosphorus**

795 Although in most instances inorganic P is considered to be the primary P-nutrient
796 for natural *Alexandrium* bloom populations, organic P compounds such as adenosine
797 triphosphate or guanosine diphosphate can increase the growth rate of some *Alexandrium*
798 species significantly (Matsuda et al., 1999). Glycerophosphate is also sometimes used as
799 a better P-source than inorganic phosphate in culture medium (Prakash, 1967; Achiha and
800 Iwasaki, 1990; but see Matsuda et al., 1999).). Low molecular weight organic-P, such as
801 phosphomonoesters, are apparently hydrolyzed to inorganic phosphate before being used
802 for growth (Gagnon et al., 2005).

803 Inorganic P uptake for *Alexandrium* has been characterized in a few studies
804 (Cembella et al., 1984; Yamamoto and Tarutani, 1999; Ou et al., 2008). Half-saturation
805 constants range from 0.01 to 2.6 μM, and were related to growth rate in *A. catenella*
806 (Jauzein et al., 2010). No multiphasic kinetics have been reported, but the range of
807 concentrations tested so far is also limited. *Alexandrium* appears to be a "storage
808 specialist" in that it can use phosphate pulses for luxury consumption and storage for
809 future use during periods of P-depletion (Yamamoto and Tarutani, 1999; Labry et al.,
810 2008).

811 **4.4 Trace metals and vitamins**

812 Early studies reported high iron (Fe) requirements for *Alexandrium* (Anderson and
813 Morel, 1979; Doucette et al., 1989). Very recently, He et al. (2010) studied the effect of

814 Fe limitation on *A. tamarense*. Growth rate and chlorophyll *a* content were reduced by
815 half, and protein by a factor of three in Fe-limited cells (1 nM Fe) relative to Fe-replete
816 controls (1 μM Fe).

817 Siu et al. (1997) studied in detail the metal requirements for *A. catenella*. The
818 optimal ranges found for cobalt, copper, iron, manganese, molybdenum, selenium and
819 zinc do not deviate significantly from the composition of commonly used culture media,
820 with the possible exception of selenium. The latter was found to be required in the range
821 20-100 nM, whereas, the concentration recommended in recent artificial seawater (e.g.,
822 ESAW) recipes is only 1 nM and 10 nM in K and L1 media supplements to natural
823 seawater (Andersen et al., 2005). Both selenium and nickel are now often added for
824 growing *Alexandrium* species, e.g., for growth of *A. fundyense* in f/2 medium with urea
825 as N-source (Taroncher-Oldenburg et al., 1997).. In contrast, other metals such as copper
826 are sometimes reduced to grow *Alexandrium* (Taroncher-Oldenburg et al., 1997), relative
827 to concentrations given for f/2 medium.

828 Vitamin requirements of *A. catenella* from Hong Kong waters (Siu et al., 1997 do
829 not deviate from those of other phytoplankton. This contrasts with a strain of *A. catenella*
830 from the South China Sea and of *A. minutum* from Rio de Vigo (Spain), which required
831 cyanocobalamine only, but neither biotin nor thiamine (Tang et al., 2010).

832 **4.5 Mixotrophy**

833 In one of the earliest studies on toxin production in *Alexandrium*, Proctor et al.
834 (1975) reported uptake of fourteen ^{14}C-labelled organic compounds by *A. catenella*, the
835 most prominently retained in cells being guanine, guanosine, formate and urea. For
836 *Alexandrium* species, there is evidence for uptake of large molecules such as dextran-

837 labeled with fluorescent markers (Legrand and Carlsson, 1998), and humic substances
838 labeled with ^{14}C (Doblin et al., 2001). Those humic substances are thought to play a role
839 as growth promoters (by complexing metals or affecting nutrient transport mechanisms)
840 rather than sources of nutrients (Gagnon et al., 2005).

841 Urea assimilation by *Alexandrium* involves the enzyme urease (Dyhrman and
842 Anderson, 2003). Urease activity was highest in N-starved and urea-grown cultures, and
843 undetectable in nitrate-grown cultures. Indirect evidence from mass balance
844 considerations indicates use of DON other than urea both in laboratory cultures (Collos et
845 al., 2004, 2006) and natural populations of *A. catenella* (Collos et al., 2007).

846 Under P deficient conditions, some *Alexandrium* species are known to produce
847 alkaline phosphatase (Oh et al., 2002; Ou et al., 2006) allowing use of organic P.
848 Although Flynn et al. (1996) could not establish alkaline phosphatase activity as an
849 indicator of phosphate stress in three *Alexandrium* species, Oh et al. (2002) and Jauzein et
850 al. (2010) reported synthesis of this enzyme below an inorganic P threshold of 0.4 – 1
851 μM in *A. tamarense* and *A. catenella*.

852 **4.6 Phagotrophy**

853 Phagotrophy is apparently widespread among *Alexandrium* species (Jeong et al.,
854 2010). Both bacteria and flagellates have been observed in food vacuoles of *Alexandrium*
855 (Jeong et al., 2004). *Alexandrium ostenfeldii* is also known as a mixotroph with
856 phagotrophic capabilities, based on examination of food vacuoles (Jacobson and
857 Anderson, 1996). In a recent review of the phagotrophic capacities of mixotrophic
858 dinoflagellates (Jeong et al., 2010), *A. minutum* was reported to ingest cyanobacteria, and
859 *A. catenella* both heterotrophic bacteria and cyanobacteria, but *A. tamarense* could also

860 ingest other prey such as haptophytes, cryptophytes, small diatoms, the raphidophyte
861 *Heterosigma akashiwo* and the dinoflagellates *Amphidinium carterae* and *Prorocentrum*
862 *minimum*.

863 **4.7 Heterogeneity in gross (or intrinsic) growth rates**

864 *Alexandrium* species will grow in a variety of media, based either on natural
865 seawater enrichments (e.g., f/2, K, L1) or artificial (e.g., AK, Aquil, ESAW) seawater.
866 Genetic variability in growth rate is extensive among *Alexandrium* species and strains
867 even when grown under standard conditions. *Alexandrium tamarense,* for example, can
868 exhibit a range of growth rates (μ) of up to 1.0 d^{-1}. Brand (1981) recorded a high range of
869 μ from 0.19 to 0.66 d^{-1} for 75 clones of *A. tamarense* grown under identical conditions,
870 whereas Costas (1990) found a larger and Tillman et al. (2009) a lower clonal variation in
871 μ. It may be significant that the highest μ ever recorded for this species was for a culture
872 incubated under natural irradiance and temperature (Smayda, 1996).

873 For *A. catenella*, the highest μ recorded in laboratory cultures was 0.55 d^{-1} (Matsuda
874 et al., 1999; Collos et al., 2004), still lower than the highest gross μ (0.89 d^{-1}) recorded
875 for monospecific blooms of the same species by the dilution method (Collos et al., 2007).
876 These discrepancies seem to point out possible inadequacies of culture media and/or
877 culture conditions relative to the natural environment, and the consequent possible
878 underestimation of growth rate of *Alexandrium* species under laboratory conditions. This
879 is important regarding, for example, the wide and long-standing debate on the relative
880 growth rates of diatoms and dinoflagellates, and determination of realistic rates for
881 parameterization of predictive bloom models.

5 TOXINS AND ALLELOCHEMICAL INTERACTIONS

The genus *Alexandrium* is notorious for the production of potent neurotoxins and other unrelated allelochemicals affecting species interactions and the health of marine fauna, as well as of human seafood consumers via paralytic shellfish poisoning (PSP). *Alexandrium* has the distinction of being the first dinoflagellate genus associated unequivocally as the source of phycotoxins affecting human health. Investigations on the cause of toxicity in shellfish established a link between *A. catenella* (referred to as *Gonyaulax catenella*) in the water column and shellfish toxicity at the Pacific coast and for *A. tamarense* (referred to as *Gonyaulax tamarensis*) at the Atlantic coast of North America (reviewed by Prakash et al., 1971). The key neurotoxic tetrahydropurine alkaloid saxitoxin (STX) was isolated and characterized from cultures of *A. catenella* (Schantz et al., 1966). The advent of liquid chromatography coupled to tandem mass spectrometry, in concert with high resolution NMR, for structural elucidation has led to the characterization of more than a dozen naturally occurring PSP toxin analogues among various *Alexandrium* species.

Although *Alexandrium* is not the unique source of PSP toxins among dinoflagellates – these toxins are also produced by *Gymnodinium catenatum* and *Pyrodinium bahamense*, as well as several genera of predominantly freshwater cyanobacteria - the wide distribution of this genus renders it the most globally important producer. In this review the multifaceted aspects related to toxigenicity of *Alexandrium* are restricted to a focus on highlights of three major issues: 1) the validity of toxin composition profiles as chemotaxonomic and phenotypic markers within and among *Alexandrium* species; 2)

biosynthesis of saxitoxin and analogues and spirolides; and 3) allelochemical interactions between and among species.

5.1 Variation in toxin content and composition

In Alexandrium, the composition of PSP toxins typically includes several members of one or more of the following sub-groups: 1) carbamoyl toxins, including saxitoxin (STX), neosaxitoxin (NEO) and the C-11 O-sulfated analogues gonyautoxins (GTX1 – GTX4) and 2) N-21 sulfocarbamoyl analogues (B1 = GTX5, B2 = GTX6, C1 – C4). *Alexandrium* strains produce different relative amounts of these derivatives, but the composition is a stable phenotypic trait and significant shifts tend to occur only under rather extreme change in growth regime in batch and semi-continuous cultures (e.g., Hall, 1982; Boyer et al., 1987; Boczar et al., 1988; Anderson et al., 1990).

The production of a certain suite of toxins seems to be fixed genetically for each clonal strain of *Alexandrium* (Anderson et al., 1990; Cembella, 1998 and references therein). Although the PSP toxin profile varies widely within and among *Alexandrium* species, general characteristics can usually serve to identify the distinction from the toxin composition of other dinoflagellate genera (*Pyrodinium* and *Gymnodinium*) and cyanobacteria, or as sequestered in shellfish. For example, in *Alexandrium* species, decarbamoyl derivatives (dcSTX, dcNEO, dcGTX1-4) and the N-21 sulfocarbamoyl analogues C3, C4 are rarely found. Within *Alexandrium*, it is sometimes but not always possible to identify species-specific toxin markers. Members of the *A. minutum* group (including also *A. ibericum*, *A. lusitanicum*, *A. angustitabulatum*) tend to produce primarily or exclusively gonyautoxins (GTX1-GTX4) (Cembella et al., 1987). Among

926 species of the *A. tamarense* complex, however, toxin profiles are too diverse to be
927 diagnostic for species discrimination.

928 Cellular toxin content is a less stable phenotypic character of a clonal isolate of
929 *Alexandrium* than its toxin profile (Cembella et al., 1987). Average cellular toxin content
930 of toxigenic *Alexandrium* isolates varies considerably (up to an order of magnitude)
931 among different growth phases and environmental regimes in batch cultures, with
932 maxima usually found in exponential phase and under P-limitation (e.g., Boczar et al.,
933 1988; Anderson et al., 1990). Furthermore, within *Alexandrium* species, clone-specific
934 toxin content can vary from undetectable to >100 fmol $cell^{-1}$, even among clones isolated
935 from the same geographical population. This implies that cell PSP toxin content is not
936 reliable as a species-, ribotype-, or population-characteristic and must be interpreted
937 cautiously. Even though distributions of toxin phenotypes of *A. minutum* appeared not to
938 overlap in Irish coastal waters, with toxic forms found in the south, and non-toxic strains
939 in the west (Touzet et al., 2008a,b), toxic and non-toxic strains of *A. minutum* cluster
940 together in phylogenetic analyses (Lilly et al., 2005). On the other hand, both toxic and
941 non-toxic phenotypes, corresponding to the Group I and Group III clades within the *A.*
942 *tamarense* complex (Lilly et al., 2007) have been documented to co-occur geographically
943 in the Shetland Islands in Scotland (Touzet et al., 2010) and Belfast Lough in Northern
944 Ireland (Brosnahan et al., 2010).

945 Investigations on PSP toxin composition of *Alexandrium* isolates and natural
946 population have interpreted toxin profiles chemotaxonomically to differentiate among
947 morphotypic and genotypic variants within and among species and geographical
948 populations (Cembella et al., 1987, Anderson et al., 1994). These early studies revealed

949 considerable inter-population variation in toxin composition not only between different
950 locations, but also among isolates within geographical populations, although
951 biogeographical trends could often be discerned. In the Gulf of Maine, the apparent
952 northward gradient of increasing cell toxicity was attributed to differences in total cellular
953 toxin content, as well as to a progressive shift in relative composition to more highly
954 toxic carbamoyl derivatives (Anderson et al*.,* 1994). Multivariate statistical techniques
955 applied to toxin composition data from regionally separated populations showed that in
956 some cases regional populations of *Alexandrium* can clearly be distinguished from others
957 by toxin profiles (Cembella et al*.,* 1987; Anderson et al*.,* 1994; Cembella and Destombe,
958 1996). Comparison of PSP toxin composition of field samples of planktonic *A. tamarense*
959 populations from different sampling sites in eastern Canada (Cembella and Destombe,
960 1996) serves to illustrate biogeographical patterns. Populations from the Bay of Fundy
961 and the St. Lawrence estuary display homogeneity in the relative amounts of PSP toxins,
962 whereas populations from Nova Scotia were characterized by larger intra-regional
963 differences in toxin composition. Based upon toxin profiles as a chemotaxonomic
964 character at the population level, this implies that the St. Lawrence populations are well
965 mixed and presumably seeded from the same cyst beds at the northern shore of the
966 estuary. Yet they are clearly distinct from the other eastern populations from Nova
967 Scotia, indicating a geographical separation that leads to reproductive isolation of
968 populations.

969 There are two possible explanations for the development of inter-population
970 differences in PSP toxin composition among *Alexandrium tamarense/fundyense* (Group I
971 ribotype) populations at the Atlantic coast of North America (Anderson et al., 1994). One

explanation is that environmental factors favor the selection of certain phenotypically differentiated individuals originating from a common cyst bed. Such locally differing selection during development of vegetative growing populations could lead to the establishment of phenotypically differentiated bloom populations after dispersal to different regions. Alternatively, dispersal of *Alexandrium* populations from different centers of origin may explain inter-population differences in the relative composition of PSP toxins. Whereas the first mechanism is based on the idea of short-term differentiation of planktonic populations, the second implies long-term processes, which might be enhanced by prevailing current patterns.

These two hypotheses were tested using microsatellite analyses of temporally and geographically separated samples from a widespread *A. fundyense* bloom in the Gulf of Maine (Erdner et al., 2011). Results indicate that *Alexandrium* blooms derive from a single regional population of *A. fundyense* comprising at least two genetically distinct sub-populations. These subpopulations were characteristic of early- and late-bloom samples and were collected from the northern and southern areas of the bloom, respectively. The presence of genotypes from both sub-populations in mid-bloom samples from north of Cape Cod, combined with drifter data on current patterns, does not support the presence of separate north and south centers of origin for the bloom. Although the definitive test of these two alternatives – determination of the genetic composition of the cyst seedbeds – remains to be done, it is most likely that *Alexandrium* blooms in this region originate from a common cyst source, congruent with the former hypothesis of Anderson et al. (1994) and the conceptual model proposed by Alpermann et al. (2009).

995 Distribution of toxin phenotypes of *A. ostenfeldii*, which may produce spirolides
996 and/or PSP toxins, may be interpreted similarly, but available data are more limited than
997 for the *A. tamarense* complex (Cembella and Krock, 2007). Nevertheless, stability of the
998 spirolide toxin profiles indicates that they may also serve as phenotypic or
999 chemotaxonomic markers. Strains of *A. ostenfeldii* from New Zealand and the Baltic Sea
1000 tend to produce exclusively PSP toxins, whereas those from Nova Scotia yield only
1001 spirolides, and some from Denmark can synthesize both toxin groups. The spirolide
1002 profiles of both isolates and field populations from the northwestern Atlantic are often
1003 heavily dominated by 13-desmethyl spirolide C (13-desmeC), but may also contain
1004 variants such as spirolide A, B, C or D-type (Cembella et al., 2000, 2001). In contrast, an
1005 isolate from the North Sea coast of Scotland yields exclusively 20-methyl spirolide G
1006 (20-meG), whereas one from the Celtic Sea contains this analogue, but also slight
1007 amounts of 13-desmeC. Multi-year samples of field populations containing *A. ostenfeldii*
1008 from the North Sea and adjacent waters consistently showed 20-meG as dominant, albeit
1009 that 13-desmeC was also often present, particularly along the Irish coast. In comparison,
1010 Mediterranean isolates contain overwhelmingly 13-desmeC. Analysis of spirolide toxin
1011 profiles from natural populations and isolates of *A. ostenfeldii* from the Gulf of Maine
1012 revealed not only the regional diversity among populations but also the presence of five
1013 distinct spirolide toxin phenotypes among isolates (Gribble et al., 2005).

1014 These examples illustrate that biosynthesis of particular toxin analogues is subject
1015 to inter- and intraspecific variation, including at the population level and even in some
1016 cases among clones within a population. In any case, the interpretation of cell toxin
1017 content and composition as phenotypic markers in natural populations of *Alexandrium* or

1018 from clonal isolates is subject to major limitations and pre-conditions that are not usually
1019 fulfilled in most studies. First, determination of cell toxin content and composition from
1020 mixed assemblages can include several *Alexandrium* taxa that may not be resolved
1021 morphologically or genetically. Second, in multiclonal populations the cell toxin content
1022 and profile can only represent the mean of the relative distribution of toxin phenotypes.
1023 Finally, often only one or a few clonal isolates are selected to represent the population
1024 without reference to genetic heterogeneity. This invokes the "genetics of survivors" and
1025 autecological dependency limitations for population studies. In one of the rare studies
1026 comparing PSP toxin variation with genetic markers for a high number of cultured
1027 isolates (88 clones), Alpermann et al. (2010) addressed this issue and showed that within
1028 a geographical population of *A. tamarense* (Group I/North American ribotype) from the
1029 North Sea, PSP toxin composition was highly heterogeneous among clones.
1030 Nevertheless, cluster analysis did reveal hierarchical grouping according to toxin profiles,
1031 but no clear linkage to molecular markers such as AFLP and microsatellites. Similar
1032 findings were obtained for *A. fundyense* populations from the Gulf of Maine (D.M.
1033 Anderson, unpub. data).

1034 **5.2 Toxin biosynthesis**

1035 The biosynthesis and gene regulation of the tetrahydropurine saxitoxin and
1036 analogues in dinoflagellates, and particularly among *Alexandrium* species, has long been
1037 the subject of intensive speculation and research interest. Based upon stable isotope
1038 precursor labeling experiments followed by NMR for both the cyanobacterium
1039 *Aphanizomenon flos-aquae* and *A. tamarense*, Shimizu (1996) proposed a unique
1040 biosynthetic pathway for saxitoxin (STX) involving arginine, acetate, and methionine as

1041 building blocks, with assembly initiated by a Claisen condensation between arginine and
1042 acetate. Characterization of putative PSP toxin biotransformation enzymes (e.g., N-
1043 sulfotransferases) from *A. catenella* and *Gymnodinium catenatum* (Ishida et al., 1998)
1044 also tended to support the proposed biosynthetic pathway for both dinoflagellates and
1045 cyanobacteria. The fact that PSP toxin profiles in *Alexandrium* exhibit a biparental
1046 inheritance pattern that is consistent with Mendelian segregation implies that expression
1047 of a specific toxin profile is regulated by nuclear genes (Sako et al., 1992).

1048 Nevertheless, until recently the nature of the saxitoxin biosynthetic genes in
1049 *Alexandrium* has remained elusive. As is the case with most large-celled free-living
1050 dinoflagellates, *Alexandrium* has a huge nuclear genome (>200 pg DNA), comprising a
1051 high number (up to about 150) of chromosomes with permanently condensed chromatin,
1052 and lacking canonical histones, but rich in modified nucleotides, and with a high G-C
1053 base pair ratio. These factors, in addition to the complexity of genes organized as tandem
1054 repeats or with multiple introns, and transcribed by a spliced-leader trans-splicing
1055 mechanism (Lin et al., 2010), have to date confounded the sequencing of the
1056 *Alexandrium* genome. An early study of *A. fundyense* based upon differential display of
1057 genes (Taroncher-Oldenburg and Anderson, 2000) following cell synchronization
1058 identified three genes, S-adenosylhomocysteine hydrolase, methionine aminopeptidase,
1059 and a histone-like protein, possibly related to PSP toxin production. More recent analysis
1060 of expressed sequence tags (ESTs), short sub-sequences transcribed from cDNA libraries,
1061 for *A. fundyense* (Hackett et al., 2005), *A. ostenfeldii* (Jaeckisch et al., 2008), and *A.*
1062 *minutum* (Yang et al., 2010) has facilitated the search for toxin biosynthetic genes. An
1063 EST library constructed for the dinoflagellate *Alexandrium minutum* (Yang et al., 2010),

combined with the application of an oligonucleotide microarray uncovered 192 differentially expressed genes between toxic and non-toxic strains. Although candidate genes for possible involvement in growth regulation and/or toxin biosynthesis were found, there were no confirmed hits for the PSP toxin biosynthetic genes as in cyanobacteria.

In contrast to the biosynthesis of PSP toxins in synchronized *A. fundyense* cells, which occurs in G1 phase of the cell cycle following a light-dependent transition (Taroncher-Oldenburg et al., 1997; Taroncher-Oldenburg and Anderson, 2000), spirolide biosynthesis in *A. ostenfeldii* is restricted primarily to the G2 phase (John et al., 2001). Stable isotope feeding experiments with *A. ostenfeldii* followed by NMR (MacKinnon et al., 2006) confirmed the biosynthesis of spirolide 13-desmethyl C as a polyketide derived from acetate units, with the imine moiety derived intact from glycine. Comparative and functional genomic analysis of an EST library of *A. ostenfeldii* (Jaeckisch et al., 2008) was successful in identifying a range of polyketide synthase (PKS) genes with high sequence conservation in respect to other dinoflagellates producing polyketide toxins, such as *Karenia brevis* (Monroe and van Dolah, 2008). Specific association of particular PKS genes with spirolide biosynthesis has not yet been confirmed.

The discovery of a saxitoxin gene cluster (*sxt*) and a biochemically determined plausible biosynthetic pathway for saxitoxin in the cyanobacterium *Cylindrospermopsis raciborskii* (Kellmann et al., 2008), and variations of the sxt cluster to account for the biosynthesis of sulfated analogues (GTXs) in other cyanobacteria (Soto-Liebe et al., 2010) dramatically accelerated the search for homologous gene clusters in *Alexandrium*. Mass sequencing of mRNA transcripts from saxitoxin-producing strains of *Alexandrium*

and several other STX-producing dinoflagellates, coupled with *in silico* transcriptome analyses and various PCR techniques, successfully identified such STX-synthesis genes (Stüken et al., 2011; Hackett et al., in press). Hackett et al. (in press) identified 265 putative homologs of 14 cyanobacterial STX synthesis genes, including all of the genes directly involved in toxin synthesis in cyanobacteria (Kellmann et al., 2008). The *Alexandrium* transcripts of the *sxtA* gene have the same domain structure as those from cyanobacterial homologs, but the dinoflagellate transcripts are monocistronic, occur in multiple copies, and contain typical dinoflagellate spliced-leader sequences. Furthermore, investigation of STX-producing and non-producing dinoflagellate strains from six different genera showed congruence for the presence of the *sxtA* gene and STX-synthesis, except for three strains of *A. tamarense*, for which *sxtA* was amplified without evidence of STX or derivatives (Stüken et al., 2011).

In spite of the fact that the basic pathway for STX biosynthesis is generally consistent with that proposed originally by Shimizu (1996) for both cyanobacteria and dinoflagellates, molecular evidence now suggests that the functional homologs of *sxtA*, *sxtG* and *sxtB* arose independently in dinoflagellates and cyanobacteria (Hackett et al., in press; Stüken et al., 2011).

5.3 Allelochemical interactions

Allelochemical activity towards potential protistan and macrozooplankton grazers and/or resource competitors has been widely documented among *Alexandrium* species. Against other protists, allelochemical effects of exposure to *Alexandrium* cells or cell-free culture medium (filtrate) of *Alexandrium* spp. typically results in immobilization of target cells followed by their lysis or cyst formation (Tillmann and John, 2002; Fistarol et al.,

2004). Addition of filtered culture medium of an allelopathic strain of *A. tamarense* to a natural plankton assemblage provoked drastic alterations in the experimental plankton community and especially a marked reduction of ciliate micrograzers. Protists shown to be sensitive to *Alexandrium* allelochemical activity include various diatoms, haptophytes, cryptophytes, chlorophytes, ciliates and even other dinoflagellates; the latter group includes both obligate autotrophic and heterotrophic as well as mixotrophic species (e.g., Hansen, 1989; Arzul et al., 1999; Tillmann and John, 2002; Tillmann et al., 2007). The potency and wide spectrum of putative targets suggests that allelochemical interactions may be highly adaptive and play an important role in *Alexandrium* bloom dynamics and ecological niche differentiation. However, allelopathic activity is not ubiquitous among *Alexandrium* populations or universally effective against all potential targets in natural plankton assemblages; even in extremely dense *Alexandrium* blooms grazing by tintinnid ciliates can contribute to bloom termination (Sorokin et al., 1996).

Neither the chemical nature of the allelochemicals nor their genetic regulation and mode of action are well understood for *Alexandrium* species. Given the frequent occurrence of saxitoxin and analogues among *Alexandrium* populations, it has long been postulated and even assumed that these potent sodium-channel blocking neurotoxins act ecologically as a classic chemical defense against grazers and competitors (reviewed by Cembella, 2003) in the "watery arms race" *sensu* Smetacek (2001). This interpretation is now considered overly simplistic or perhaps even generally inaccurate for *Alexandrium*.

Studies of various copepod species grazing upon *Alexandrium* species and isolates differing in PSP toxin content and composition have yielded widely diverging responses. The differential responses among copepods range from loss of swimming coordination

and physiological incapacitation through toxin-dependent differential grazing, chemically mediated avoidance and post-ingestion rejection behavior, to no apparent relationship between cellular composition of PSP toxins and grazing behavior, grazer mortality or fecundity (reviewed by Turner et al., 1998). Thus the presence of a universal defense mechanism against copepods linked directly to PSP toxin content or composition of *Alexandrium* cells appears not to be sustainable.

Lytic allelochemical activity of selected strains of *Alexandrium* spp. towards a wide variety of both photoautotrophic and heterotrophic protists was apparently unrelated to the cellular PSP toxin content (Tillmann and John, 2002). Further experiments with multiple clones of *A. tamarense* from the Scottish east (Tillmann et al., 2009) showed high clonal heterogeneity in lytic potency against the cryptophyte *Rhodomonas salina* and the heterotrophic dinoflagellate predator *Oxyrrhis marina*, but without obvious association to cellular PSP toxin content or composition. These results indicate that PSP toxins are not the primary allelochemical in *Alexandrium* and may not be crucial in determining outcomes of competitive or grazing interactions among protists in natural assemblages.

In the first experiments on grazing interactions of *A. ostenfeldii* and the tintinnid *Favella ehrenbergii* the presence of PSP toxins (albeit as very low cellular levels) was proposed as possible waterborne cues to account for the threshold-dependent retrograde swimming behavior and grazing inhibition of the tintinnid (Hansen et al., 1992). Yet other experiments with this tintinnid exposed to multiple clones of *A. tamarense* (Hansen, 1989) that varied widely in PSP toxin content failed to show a relationship of these toxins to tintinnid growth or behavior. The later discovery of spirolides in the isolates of *A.*

ostenfeldii used in the tintinnid experiments (Hansen,1989; Cembella et al., 2000, 2001) suggested that spirolides were acting as allelochemicals. This possible linkage was disproved, however, in experiments with *A. ostenfeldii* strains exposed to a wide variety of heterotrophic and phototrophic protists, which showed that lytic activity was independent of spirolide content (Tillmann et al., 2007).

The potent immobilization and lytic activity of *Alexandrium* allelochemicals against protistan cells appears to target external cell membranes (Ma et al., 2009). Furthermore, it is now clear that this activity is not mediated primarily (if at all) by known low molecular weight phycotoxins. In fact, it appears likely that a complex of allelochemicals, as originally suggested by Arzul et al. (1999) and/or high molecular weight (perhaps macromolecular) components may be involved. Most recent evidence indicates that lytic compounds from *A. tamarense* increase permeability of the cell membrane for Ca^{2+} ions, but do not specifically bind to these ion channels or cause non-specific lysis of target membranes by detergent-like activity (Ma et al., 2011). Furthermore, although the molecular targets of the lytic compounds are likely to involve sterol components of membranes, the high molecular weight (between 7 kDa and 15 kDa) precludes a direct analogy to the mode of action of karlotoxins.

Other allelochemicals of the genus *Alexandrium* include a heat-labile exotoxin from *A. minutum* (Lush et al., 2001) with potent toxicity towards the brine shrimp *Artemia salina*. A hemolytic exotoxin with a molecular weight >10 kDa and described as proteinaceous has been isolated from *A. taylori* (Emura et al., 2004), and a novel high molecular weight (about 1,000 kDa) hemolytic and cytotoxic compound, most likely polysaccharide-based, was reported from *A. tamarense* (Yamasaki et al., 2008).

1179 Nevertheless, different chemical and physical properties and the apparent lack of either
1180 polysaccharide or proteinaceous components in the structure of the lytic allelochemicals
1181 from *A. tamarense* that are effective against other protists suggest that these compounds
1182 are not related.

1183 Allelochemicals may be produced by *Alexandrium* as effectors of other species, or
1184 transduced by *Alexandrium* cells to elicit targeted behavioral and gene expression
1185 responses. In the latter case, *A. tamarense* cell chains were shown to reduce encounter
1186 rates with grazers by splitting into single cells or shorter chains and slowing down
1187 swimming speed when exposed to waterborne copepod cues (Selander et al., 2011).
1188 Naturally occurring concentrations of copepods may provoke a >25-fold increase in cell
1189 PSP toxin content in *Alexandrium minutum*, which has also been shown to correlate with
1190 increased resistance to copepod grazing (Selander et al., 2006). Waterborne cues of
1191 copepods induce change in both cell PSP toxin content and gene expression profiles in
1192 *Alexandrium* spp. (e.g., Wohlrab et al., 2010; Yang et al., 2011). In a transcriptomic
1193 model study of copepod-induced shift-up in cell PSP toxin content in *A. minutum* based
1194 upon a DNA microarray (Yang et al., 2011), a limited set of 14 genes were differentially
1195 regulated by exposure to water borne cues from copepods. Exposure of *A. tamarense* to
1196 three copepod species (*Calanus helgolandicus, Acartia clausii, and Oithona similis*) and
1197 their corresponding waterborne cues also substantiated the potential for a rapid increase
1198 in PSP toxin content in the dinoflagellate (Wohlrab et al., 2010). This functional genomic
1199 approach indicated that regulation of serine/threonine kinase signaling pathways has a
1200 major influence in directing the copepod-cues into different intracellular cascades and
1201 networks in *A. tamarense*. Bidirectional allelochemical interactions provide a plausible

basis for co-evolutionary mechanisms between *Alexandrium* and its predators and competitors in natural bloom populations.

6 BLOOM DYNAMICS

6.1 General mechanisms

The complexities of *Alexandrium* blooms in dynamic coastal or estuarine systems are far from understood. One common characteristic of such blooms is that the coupling between physics and biological "behavior" such as swimming, vertical migration, or physiological adaptation holds the key for understanding these phenomena, yet this is perhaps where our knowledge of this genus is weakest.

Once vegetative cells enter the water column following cyst germination, their net growth and transport are heavily affected by circulation, nutrients, stratification, and other chemical or physical factors (see also biological loss terms below). Although many of these interactions remain uncharacterized, blooms of several *Alexandrium* species have been linked to particular water masses. There are many examples of the importance of fronts in HAB bloom dynamics. For example, patterns of PSP toxicity and *A. tamarense* cell distributions in the lower St. Lawrence estuary have been linked to the plume produced by the Manicouagan and Aux-Outardes rivers (Therriault et al., 1985). The trans-estuarine freshwater plume generates a highly stratified water column that favors proliferation and retention of vertically migrating *Alexandrium*. The frontal system generated by the Manicouagan and Aux-Outardes plume serves as an initiation zone and the Gaspé current as a transport pathway along the south shore. The physical system is not the entire story, however. Although the riverine plume is essential for *A. tamarense*,

this species is most abundant during mid- to late-summer, even though the characteristics of the plume and the front are well-established for a much longer interval. Clearly, other factors are regulating *A. tamarense* dynamics. Therriault et al. (1985) suggested that *A. tamarense* blooms in the St. Lawrence develop only when the proper combination of meteorological and hydrodynamic factors coincide to produce high surface water temperatures, maximum water column stability, low nutrients, and low winds. These dynamics have been explored in a bloom modeling study of the region by Fauchot et al. (2008).

Another example of the importance of physical forcings in *Alexandrium* bloom dynamics is in the Gulf of Maine, where the temporal and spatial pattern of persistent PSP outbreaks have been linked to a large-scale coastal current system that traverses the Gulf (Franks and Anderson, 1992; Anderson et al., 2005a,d). Conceptual models of *A. fundyense* bloom dynamics (Anderson et al., 2005c; McGillicuddy et al., 2005) include key features such as two large cyst "seedbeds"- one in the Bay of Fundy and the other offshore of mid-coast Maine. Cysts germinate from the Bay of Fundy seedbed, causing recurrent coastal blooms in the bay that are self-seeding with respect to future outbreaks in that area. The blooms also contribute to populations in the eastern section of the Gulf as some cells escape the Bay of Fundy and enter the eastern segment of the Maine coastal current (EMCC) where they form blooms. Some *Alexandrium* cells travel south and west with that current, while others are deposited as cysts in the mid-coast Maine seedbed. In subsequent years, these latter cysts (combined with cells from the EMCC) inoculate blooms that cause toxicity in western portions of the Gulf and possibly offshore waters as well.

Another important unknown in the coastal blooms concerns the possible stimulation of *Alexandrium* growth by the unique chemistry of freshwater plumes. More *Alexandrium* cells are typically found within rather than outside the low salinity plumes (e.g., Therriault et al., 1985; Franks and Anderson, 1992), but this could be a result of small-scale physics interacting with the cells migration behavior, or a reflection of higher growth rates within the plume. Freshwater runoff from the heavily forested watershed of the Maine coast contains significant levels of dissolved and particulate organic matter as well as metals and other micronutrients. Some components of this mixture could be critical to the rapid growth of *Alexandrium* cells. Iron is a likely candidate for a stimulatory micronutrient, as Wells et al. (1991) showed that bioavailable iron was elevated in nearshore waters characteristic of the coastal current, and depleted offshore in the Gulf of Maine. The measured iron levels were within the range of those that stimulated or limited *A. tamarense* growth in laboratory cultures.

The large number of *Alexandrium* species involved in HAB events throughout the world makes it difficult to generalize about environmental controls of bloom dynamics. The nutrition of these organisms is not unusual, although mixotrophy has been reported for some *Alexandrium* species (Jacobson and Anderson, 1996; Legrand and Carlsson, 1998) and more are probably capable of this strategy. Like most phytoplankton, *Alexandrium* species will respond to anthropogenic nutrient inputs, but there is no evidence that they are preferentially stimulated compared to other phytoplankters, nor is there compelling evidence of any increase in *Alexandrium* bloom magnitude or frequency as a direct result of pollution or massive nutrient enrichment. Indeed, *Alexandrium*

1269 blooms, including many toxic ones, occur in remote and relatively pristine waters, such
1270 as those in Alaska (Hall, 1982) or southern Argentina (Benavides et al., 1995).

1271 One generalization on the dynamics of *Alexandrium* populations in shallow
1272 embayments is that such blooms are heavily dependent upon local hydrographic
1273 conditions and the manner in which these factors interact with cell behavior, especially
1274 cyst germination, and vertical migration of vegetative cells. Studies of *A. minutum* in a
1275 Mediterranean lagoon by Giacobbe et al. (1996) demonstrated that the spring appearance
1276 of the species coincided with enhanced rainfall and freshwater runoff, and with
1277 stabilization of the water column. Watras et al. (1982) conducted laboratory growth
1278 studies and used the results to parameterize a simple model, indicating that for Cape Cod
1279 salt ponds, the development of *Alexandrium* populations depends solely on salinity-
1280 dependent temperature regulation of cell division rates. The same model, however,
1281 produced a poor prediction of *Alexandrium* bloom dynamics from the Bay of Fundy,
1282 presumably because physical forcings are more influential in population accumulation in
1283 such open, tidally stirred waters.

1284 Another example of physical/biological coupling and the importance of
1285 stratification and cell swimming behavior in embayments was observed in Salt Pond, a
1286 small embayment with a shallow entrance sill that restrict outflowing water to the low
1287 density surface layer (Anderson and Stolzenbach, 1985). Diel vertical migration of *A.*
1288 *fundyense* kept cells below that depth during the night, and even when the cells migrated
1289 close to the surface during the day, they remained deep enough to avoid transport out of
1290 the embayment with the outflowing surface layer. A density-driven exchange mechanism
1291 rapidly flushes water from these salt ponds, but the residence time of the *Alexandrium*

cells is much longer due to the limited vertical extent of the migration. This coupling between organism behavior and the hydrography of the system restricts the extent to which vegetative cells and cysts can colonize adjacent waters and allows *Alexandrium* populations to accumulate to cell concentrations generating high toxicity in shellfish.

The duration of the blooms that have been followed in bays and salt ponds is generally two to three months or less (e.g., Anderson et al., 1983; Han et al., 1992). Giacobbe et al. (1996) describe an *A. minutum* bloom in a Mediterranean lagoon over a six month period, but the cell concentrations were at bloom levels for only two months in spring. In Cape Cod, most *Alexandrium* blooms develop at water temperatures that are non-optimal for rapid growth of vegetative cells. Perch Pond isolates of *Alexandrium* grow fastest at 15-20 ^{o}C in the laboratory, but once the water reaches those temperatures in the field, blooms are typically on the decline and new cysts are already falling to the sediments (Anderson et al., 1983). Similarly, Han et al. (1992) found that *A. tamarense* disappears from the water column of Chinhae Bay, Korea at temperatures well below those that support optimal growth in the laboratory. The implication is that the induction of sexuality precludes the long-term persistence of vegetative *Alexandrium* cells in the plankton.

Laboratory studies suggest that the induction of sexuality in *Alexandrium* occurs as a result of nutrient limitation, yet this is not well supported by field measurements. One problem in this regard is that gametes are not easily distinguished from vegetative cells in natural populations, and fusing gametes, though distinctive, are rarely observed. Gametes of *Alexandrium* species have thus never been enumerated in field studies. However, it is possible to recognize duplet cells as well as large, darkly-pigmented

1315 planozygotes (Anderson, 1980) and to tabulate their abundance through time. Only two
1316 studies have attempted to enumerate *Alexandrium* planozygotes during blooms in order to
1317 quantify the importance of encystment in bloom decline (Anderson et al., 1983; Takeuchi
1318 et al., 1995). Both show that sexuality is induced well before the bloom peaks, and that
1319 during this late stage of bloom development, planozygotes can comprise 20-40% of the
1320 motile population. This underestimates the total percentage of cells that become cysts,
1321 however, since it cannot account for the dynamic nature of the zygote sub-population.
1322 Each day, some planozygotes fall to the sediments as cysts, but new planozygotes appear
1323 following gamete fusion. The estimates do suggest that a large fraction of the bloom
1324 population encysts, and thus that bloom decline may be linked more to life cycle
1325 transitions than to grazing or other loss factors.

1326 Studies in three Cape Cod salt ponds over two bloom seasons demonstrated that
1327 planozygote formation did not coincide with an obvious decrease in ambient nutrients
1328 (Anderson et al., 1983). In fact, planozygotes in the plankton and new cysts at the
1329 sediment surface were first observed when external nutrients were at or above
1330 concentrations equivalent to those measured during the earlier stages of bloom
1331 development when vegetative growth was rapid. It may be that as the ambient
1332 temperature increased during the blooms, the rates of uptake and metabolism of nutrients
1333 increased as well. Thus nutrient concentrations that were sufficient for balanced (but
1334 slow) growth at colder, early-bloom temperatures may not have been sufficient to
1335 maintain balanced growth when waters warmed and the *A. tamarense* growth rate
1336 increased. A gradual decrease in internal nutrient pools would thus occur, leading to

nutrient limitation. Alternatively, other factors may regulate sexuality and cyst formation, such as cell density dependence similar to quorum sensing.

Thau Lagoon on the Mediterranean coast of France is another area where *Alexandrium* bloom dynamics have been intensively studied. Blooms of *A. catenella* are common, but water temperature must be around 20°C, and a period of calm weather is necessary. Thus blooms occur either in spring or fall, but major wind events will suppress them. Water temperature is probably a proxy for other variables such as turbulence, since dinoflagellates, including *A. catenella*, are very sensitive to agitation (Therriault et al., 1985). Unlike diatom blooms that are closely related to rain events and flash floods leading to nutrient inputs through the watershed into Thau lagoon, *A. catenella* does not necessarily bloom following a rain event and can even bloom following three weeks of dry weather. Thus, this species probably relies either on dissolved organic matter produced by diatom blooms (Loureiro et al., 2009) or particulate organic matter from picocyanobacteria (Collos et al., 2009).

From a long-term perspective, *A. catenella* blooms in Thau lagoon appear to follow a period of oligotrophication, characterized by a steady decline in soluble reactive phosphorus over 30 years (summer values range from about 1 - 10 µM and winter values from 3 µM to undetectable at present; Collos et al., 2009). This is consistent with observations in the Seto Inland Sea of Japan where blooms of *Alexandrium* species increased following reduction in nutrient inputs (e.g., Imai et al., 2006). Quoting from Anderson et al. (2002): “as the waters became less eutrophic and large biomass blooms decreased, there was a shift in species composition, leading to a greater prevalence of some that are responsible for shellfish poisonings in humans, such as *Alexandrium*

tamarense and *A. catenella*". Given the opportunistic behavior of *Alexandrium* species with respect to limiting nutrient acquisition, their blooms may be independent of eutrophic processes as defined from "classical" dissolved inorganic concentrations only.

6.2 Loss terms

Investigation of biological loss terms is a critical but often underrepresented component of attempts to understand and predict *Alexandrium* bloom dynamics. In Thau lagoon, evidence for biological loss terms include microzooplankton grazing rates that can match gross growth rates of *A. catenella* (Collos et al., 2004, 2007). In nearby Tarragona harbor in Spain (Garcés et al., 2005), microzooplankton grazing was not considered to be the main cause of *A. catenella* bloom termination. Other loss terms likely included cell lysis, microbial infection by viruses or bacteria, parasite attack, and encystment (Garcés et al., 2005).

Jeong et al. (2010) reviewed ingestion and clearance rates of copepods on *Alexandrium* spp. among other mixotrophic flagellates. Macrozooplankton grazing is generally thought to be much less important than microzooplankton grazing in regulating populations of *A. minutum* (Calbet et al., 2003). Non-toxic *A. tamarense* as well as toxic *A. catenella* are found to be excellent prey for the ciliate *Favella* spp. (Jeong et al., 2010). Among other predators, heterotrophic and mixotrophic dinoflagellates are also known to feed readily upon *Alexandrium*.

Very little is known about interaction between viruses and *Alexandrium* species. Loureiro et al. (2009) mention virus densities between 30 - 80 x 10^9 cells L^{-1} in cultures of *A. catenella* and these were thought to keep the bacterial population from becoming

dominant. Viral linkages to *Alexandrium* growth and mortality remain one of the major unknowns in the ecology of this genus.

The parasitic dinoflagellate *Amoebophrya* and the perkinsozoan flagellate *Parvilucifera* are both known to infect *Alexandrium* spp. (reviewed by Salomon and Imai, 2006). The latter parasite has now been found to infect the mobile zygote or the pellicle cyst of *A. minutum*, but not the thick-walled resting cyst or hypnozygote (Figueroa et al., 2008b). In addition, strain-specific host resistance to *P. sinerae* was documented for *A. minutum* (Figueroa et al., 2010). In Northern Britanny, *Amoebophrya* was shown to regulate populations of *A. minutum* (Chambouvet et al., 2008; Montagnes et al., 2008). Variables such as turbulence appear to reduce parasite infection for *A. minutum* (Llaveria et al., 2010). Infestation by *Amoebophrya* was also shown to be a major factor contributing to the decline of two blooms of *A. catenella* blooms in Puget Sound, Washington, USA (Nishitani et al., 1984). Although it has not been well documented with empirical data, parasites could be more important than microzoplankton as loss factors (Montagnes et al., 2008).

7 Modeling

Models of various types have been developed to describe and investigate physiology, toxicity, and bloom dynamics of *Alexandrium*. At the physiological level, growth and PSP toxin content of *A. fundyense* was modeled by John and Flynn (2002) from data for ammonium- and nitrate-grown cultures that were either P-replete or P-stressed. The model demonstrated a good fit to almost all cellular quota data and allowed the authors to examine the consequences of recycling toxin-N, versus not producing toxins at all. These calculations suggested that there may not be a specific evolutionary

advantage to toxin production, possibly explaining the significant variability in PSP toxin synthesis capabilities within the genus *Alexandrium*.

An extension of this model (Flynn, 2002) simulated PSP toxin content for *A. fundyense* in response to N and P nutritional status within a vertical water column in which the organism migrated. Growth in an N-limited water column resulted in a continual (although low level) toxin production with a large population biomass. A sequence of P-stress and nutrient re-feeding events during vertical migration showed an enhancement of PSP toxin content even with only moderately elevated N:P ratios. Although the final biomass was lower in these P-limited simulations, total toxin production was much higher. Vertical migration in stratified waters with moderately high N:P conditions could thus result in the formation of highly toxic populations of *Alexandrium*.

The role of resting cysts on the development of *A. minutum* blooms in a typical Mediterranean semi-enclosed water body (Arenys de Mar Harbor, NW Mediterranean) was studied by means of matrix and dynamic population models (Estrada et al., 2010). A series of scenarios were tested to determine whether excystment and encystment fluxes and changes in the dormancy period had a major effect on bloom intensity and duration. The results highlighted the importance of knowing not only the magnitude and variability of growth and life-cycle transition rates, but also those of loss rates (both in the water column and in the sediment) due to physical or biological factors. Given the maximum but low encystment rates determined for *A. minutum* in the study area (0.01 d^{-1}), this process reduced the peak concentrations of vegetative cells but did not have a major effect on bloom termination. Excystment fluxes could enhance population densities of

vegetative cells during times of low or negative net growth rate and during the initial phases of a bloom. Once exponential growth began, however, additional excystment had a negligible effect on bloom magnitude. More complex models will be needed to explore the implications of different life-cycle strategies in a wider natural ecological context.

Models have also been developed to simulate *Alexandrium* population dynamics in the field. These typically have two components – a hydrographic model, and a biological submodel. This type of physical–biological model was developed for *A. tamarense* blooms in the lower St. Lawrence estuary in eastern Canada in order to explore the interactions between cyst germination, cellular growth and water circulation and to identify the effect of physical processes on bloom development and transport across the estuary (Fauchot et al., 2008). The biological model was parameterized using an observed *A. tamarense* cyst distribution, cyst germination rate and timing, and *A. tamarense* growth limitation by temperature and salinity. The model successfully reproduced the timing of the *A. tamarense* bloom in 1998, its coincidence with the combined plumes from two rivers on the north shore of the estuary, and the temporal variations in the north-south gradients in cell concentrations.

Another well-developed physical-biological model is that used to investigate *A. fundyense* and PSP dynamics in the Gulf of Maine (e.g., McGillicuddy et al., 2005). This model is based on a hydrographic submodel that can realistically simulate water motion over this large region, as driven by winds, tides, stratification, river run off, and large-scale forcing from the open ocean. A second submodel is then coupled to the hydrography, simulating the germination of *Alexandrium* cysts from seed beds in the region, and the subsequent growth of the population, regulated by temperature, salinity,

sunlight and nutrients. The timing and rates of cyst germination and cell growth are parameterized from laboratory experiments on cultures of *A. fundyense* (Stock et al., 2005). A temperature-dependent mortality function incorporates a range of loss factors, including grazing and encystment. This model has demonstrated high fidelity at reproducing observations (Stock et al., 2005; He et al., 2008) and thus has been heavily used for hindcasts (looking at past events to understand underlying mechanisms; He et al., 2008; Li et al., 2009). The model is also being used to issue weekly nowcasts and forecasts (looking forward 3 or 4 days) and even seasonal or annual forecasts (McGillicuddy et al., in press).

8 OVERVIEW AND SUMMARY

The ability of *Alexandrium* species to colonize multiple habitats and to persist over large regions through time is testimony to the adaptability and resilience of this important organism. *Alexandrium* species are not known for rapid or "explosive" growth rates. At large spatial scales (>100 km), population growth is typically not reflected in monospecific blooms but rather in moderate biomass levels and co-occurrence with other species. Blooms are not particularly long-lasting (days to weeks), and seem restricted in time by life cycle transitions. The cyst stage is clearly important in the population dynamics of many *Alexandrium* species, but the nature of this linkage varies among habitats. In shallow embayments, cysts and motile cell blooms are tightly coupled, whereas in large temperate estuaries and open coastal waters, the linkage is more difficult to define and quantify. In both of these habitats, most of the cysts in the sediments do not germinate due to bioturbation, burial, and inhibition of germination by anoxia. Even when only the cysts in surface sediments are considered, the bulk of the widely

distributed cysts in deeper waters may germinate too slowly or too far from suitable growth conditions to be a factor in coastal blooms.

Estimates of the inoculum size from excystment are small - on the order of tens to hundreds of cells per liter, suggesting that major blooms require multiple, sustained vegetative divisions that in turn depend greatly on environmental conditions affecting motile cells. Nevertheless, the size of an excystment inoculum can have a bearing on the magnitude of a bloom, especially if that bloom is limited temporally due to seasonal temperatures or to some form of endogenous regulation of excystment and encystment.

In small-scale blooms in embayments and in widespread coastal blooms, physical/biological coupling is a critical feature of population accumulation, growth, and dispersal. Behavioral adaptations such as vertical migration are important features in this regard. Bloom termination is clearly linked to life cycle transitions, although the relative importance of encystment relative to grazing or other loss factors has not been sufficiently investigated.

In one sense, *Alexandrium* species appear to use a type of r-selection strategy, producing many "offspring" in the form of cysts, only a few of which ever germinate to inoculate blooms. On the other hand, a complex life history and a low growth rate are often considered *K*-strategies. The production of toxins and other allelochemicals to mediate inter-specific interactions is also more typical of the latter adaptive strategy. This group of dinoflagellates does not therefore easily fit into such fixed categories.

Overall, the *Alexandrium* species that have been studied in detail have proven to be remarkably resilient and capable of colonizing a wide spectrum of habitats and hydrographic regimes. It is thus of no surprise that the biogeographic range of these

1497 species has expanded in recent times and that associated PSP outbreaks remain a
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2416 **Tables**

2417

2418 Table 1. Morphotaxonomic assignments and toxicity among *Alexandrium* species. Toxin production may be highly inconsistent and
2419 therefore toxigenicity is reported only when at least one strain of the species is known to produce the designated toxin. (*) species for
2420 which a detailed description accompanied with drawings is available in Balech (1995). (§) species assigned to the subgenus
2421 *Gessnerium*. (#) marks additional references that might be considered for species identification and/or for the clarification of their
2422 taxonomy.

Species	**Basionyms/Synonyms**	**First description**	**Toxin type**	**Comments**
*Alexandrium acatenella** (Whedon & Kofoid) Balech	*Gonyaulax acatenella* Whedon & Kofoid *Protogonyaulax acatenella* (Whedon & Kofoid) Taylor *Gessnerium acatenellum* (Whedon	Whedon and Kofoid (1936)	saxitoxins	toxin type assumed only from mouse bioassay symptoms of shellfish toxicity associated with blooms

	& Kofoid) L.Loeblich & Loeblich III			
*Alexandrium affine** (Inoue & Fukuyo) Balech	*Protogonyaulax affinis* Inoue & Fukuyo *Alexandrium fukuyoi* Balech	Fukuyo et al. (1985)	saxitoxins	typically low toxicity or non-toxic
Alexandrium andersonii Balech*		Balech (1990)	saxitoxins	most commonly non-toxic
*Alexandrium angustitabulatum** Taylor	possible synonym of *A. minutum*	Balech (1995) (Hansen et al 2003)	saxitoxins	strains from the type locality weakly toxigenic
*Alexandrium balechii**§ (Steidinger) Balech	*Gonyaulax balechii* Steidinger *Gessnerium balechii* (Steidinger) Loeblich III & Loeblich, 1979	Steidinger (1971)	none known	blooms coincident with mass fish mortalities in type locality probably due to oxygen depletion

	Pyrodinium balechii (Steidinger) Taylor, 1976			
Alexandrium camurascutulum MacKenzie & Todd		MacKenzie and Todd (2002)	none known	
*Alexandrium catenella** (Whedon & Kofoid) Balech	*Protogonyaulax catenella* (Whedon & Kofoid) Taylor *Gessnerium catenellum* (Whedon & Kofoid) Loeblich & Loeblich *Gonyaulax catenella* Whedon & Kofoid	Whedon and Kofoid (1936)	saxitoxins	
*Alexandrium cohorticula**	*Gonyaulax cohorticula*	Balech (1967)	saxitoxins	Japanese strains

(Balech) Balech	Balech *Protogonyaulax cohorticula* (Balech) Taylor *Gessnerium cohorticula* (Balech) L. Loeblich & Loeblich III			reportedly toxigenic, but possible misidentification of *A. tamiyavanichii*
*Alexandrium compressum** (Fukuyo, Yoshida & Inoue) Balech	*Protogonyaulax compressa* Fukuyo, Yoshida & Inoue	Fukuyo et al. (1985)	none known	
Alexandrium concavum§* (Gaarder) Balech emend. Larsen & Nguyen-Ngoc	*Goniodoma concavum* Gaarder	Gaarder (1954) Larsen and Nguyen-Ngoc (2004)#	none known	
Alexandrium foedum§* Balech		Balech (1990)	none known	

*Alexandrium fraterculus** (Balech) Balech	*Gonyaulax fratercula* Balech *Gessnerium fraterculum* (Balech) Loeblich & Loeblich III *Protogonyaulax fratercula* (Balech) Taylor	Balech (1964)	none known	
*Alexandrium fundyense** Balech		Balech (1985)	saxitoxins	
Alexandrium gaarderae Nguyen-Ngoc & Larsen	*Gonyaulax concava* (Gaarder) Balech *Alexandrium concavum* (Gaarder)	Larsen and Nguyen-Ngoc (2004)	none known	

	Balech			
Alexandrium globulum§ Nguyen-Ngoc & Larsen		Larsen and Nguyen-Ngoc (2004)	none known	
*Alexandrium hiranoi**§* Kita & Fukuyo	*Goniodoma pseudogoniaulax* Biecheler *sensu* Kita, Fukuyo, Tokuda & Hirano (1985)	Kita and Fukuyo (1988)	goniodomins	
*Alexandrium insuetum**§* Balech		Balech (1985)	none known	
*Alexandrium kutnerae** (Balech) Balech	*Gonyaulax kutnerae* Balech	Balech (1979)	none known	
*Alexandrium leei** Balech		Balech (1985)	none known	typically non-toxic, but low level of saxitoxin derivative reported from

				Vietnamese strain; unknown ichthyotoxins
Alexandrium margalefii§ Balech		Balech (1994)	none known	
*Alexandrium minutum** Halim	*Alexandrium ibericum* Balech *Alexandrium lusitanicum* Balech *Pyrodinium minutum* (Halim) Taylor	Halim (1960) Balech (1989)#	saxitoxins	non-toxic strains also occur, e.g. in the Mediterranean Sea
Alexandrium monilatum§ (Howell) Balech	*Gonyaulax monilata* Howell *Gessnerium mochimaensis* Halim *Gessnerium monilata*	Howell (1953)	goniodomins	strongly ichthyotoxic

	(Howell) Loeblich III *Pyrodinium monilatum* (Howell) Taylor			
*Alexandrium ostenfeldii** (Paulsen) Balech & Tangen	*Goniodoma ostenfeldii* Paulsen *Goniaulax ostenfeldii* (Paulsen) Paulsen *Heteraulacus ostenfeldii* (Paulsen) Loeblich III *Gessnerium ostenfeldii* (Paulsen) Loeblich III & L.A. Loeblich *Triadinium ostenfeldii* (Paulsen) Dodge	Paulsen (1904) Balech and Tangen (1985)#	spirolides; saxitoxins	strains tend to produce either saxitoxins or spirolides, but rarely both groups

	Pyrodinium phoneus Woloszynska & Conrad *Goniaulux tamarensis* var. *globosa* Braarud *Gonyaulax globosa* (Braarud) Balech *Gonyaulax trygvei* Parke *Protogonyaulax globosa* (Braarud) Taylor			
*Alexandrium peruvianum** (Balech & Mendiola) Balech & Tangen	*Gonyaulax peruviana* Balech & Mendiola	Balech and Mendiola, 1977	spirolides	spirolides produced by strains from the Mediterranean Sea

*Alexandrium pseudogonyaulax**§ (Biecheler) Horiguchi ex Yuki & Fukuyo	*Goniodoma pseudogonyaulax* Biecheler	Biecheler (1952)		
*Alexandrium satoanum**§ Yuki & Fukuyo		Yuki and Fukuyo (1992)		
*Alexandrium tamarense** (Lebour) Balech	*Gonyaulax tamarensis* Lebour *Gessnerium tamarensis* (Lebour) Loeblich III & A.L. Loeblich *Protogonyaulax tamarensis* (Lebour) F.J.R.Taylor *Gonyaulax tamarensis*	Lebour (1925)	saxitoxins	non-toxic strains also occur; undefined allelochemicals/ ichthyotoxins may be produced

	var. *excavata* Braarud *Gonyaulax excavata* (Braarud) Balech *Protogonyaulax excavata* (Braarud) F.J.R.Taylor *Alexandrium excavatum* (Braarud) Balech & Tangen			
*Alexandrium tamiyavanichii** Balech		Balech (1994)	saxitoxins	
Alexandrium tamutum Montresor, Beran & John		Montresor et al. (2004)	none known	
Alexandrium taylori§* Balech		Balech (1994)	saxitoxins	usually non-toxic, but also known to produce

				non-proteinaceous exotoxin
*Alexandrium tropicale** Balech		Balech (1971)	none known	

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2424

2425 **Table 2: Primer sequences for ribosomal RNA genes of *Alexandrium* species**

Target gene/marker	**Target taxa**	**Primer name**	**5'-3' Sequence**	**Reference**
28S rRNA	Dinophyceae	D1R D2C	ACCCGCTGAATITAAGCATA CTTGGTCCGTGTTTCAAGA	Scholin et al., 1994
28SrRNA	*Alexandrium* species	Alex1(r)	ACCACCCACTTTGCATTCCA	Guillou et al., 2002
	Alexandrium catenella (TA clade)	Acat1(r)	GCACTACAATCTCACTGAGG	
	Alexandrium catenella (NA clade)	Acat3(r)	AAGTGCAACACTCCCACCAA	
	Alexandrium minutum	Amin2(r)	Amin2 AGCACTGATGTGTAAGGGCT	
	Alexandrium fundyense	(f)	GAATGCAAAGTGGGTGG	Dyhrman et al., 2006
28S rRNA D1/D2	*Alexandrium* *tamarense*	Atama-F3 Atama-R1	ACCTTTGCACATGAATGATAAGTC CATCCCCAAGCACAGGAAC	Nagai, 2011
	Alexandrium catenella	Acat-F3 Acat-R2	CAAAGTAAACAGACTTGATTTCCTC GAAAGCAACCTCAAGGACAAG	

	Alexandrium fraterculus	Afra-F1 Afra-R3	GCTTTGAATTGTGTTTGTGAAC GTCAGTGTTAAAGCTTGTGGG	
	Alexandrium pseudogoniaulax	Apseu-F2 Apseu-R2	GGGTGGTAAATTTCACGCAAG TGGCAACAGCTGACAATCGCA	
18S rDNA	*Alexandrium monilatum*	1F 1800R	AACCTGGTTGATCCTGCCAGT TCCTTCTGCAGGTTCACCTAC	Rogers et al., 2006
	Alexandrium catenella	Acat-F3 Acat-R2	CAAAGTAAACAGACTTGATTTCCTC GAAAGCAACCTCAAGGACAAG	
ITS1-5.8S-ITS2				
ITS1–5.8S–ITS2	*Alexandrium*	ITSA ITSB	CCTCGTAACAAGGCTCCGTAGGT CAGATGCTAAGTTCAGCA	Adachi et al., 1994
ITS1–5.8S–ITS2	*Alexandrium*	P1 P2	GTAGGATCCGGTGAACCTTGCAGAAGGA ATCGAATTCCTCCGCTTACTTATATGC	Spalter et al., 1997
	Alexandrium	5.8S-b5' 5.8S-b3'	YGATGAAGAATGCAGCAAMATG CAAGCAHACCTTCAAGMATATCC	Galluzzi et al., 2004
ITS1–5.8S–ITS2	*Alexandrium*	5.8S-5'	GCAADGAATGTCTTAGCTCAA	Galluzzi et al., 2005

	Alexandrium minutum	ITS1m (f) 5.8S-3'	CATGCTGCTGTGTTGATGACC GCAMACCTTCAAGMATATCCC	
ITS1–5.8S–ITS2	*Alexandrium andersonii*	5.8S-5' ITS2an	GCAADGAATGTCTTAGCTCAA GATGACACGTTTCGGCAAG	Penna et al., 2007
	Alexandrium catenella	ITS1c 5.8S-3'	AGCATGATTTGTTTTTCAAGC GCAMACCTTCAAGMATATCCC	
	Alexandrium tamarense	5.8S-5' ITS2t	TGTTACTTGTACCTTTGGGA ACAACACCCAGGTTCAAT	
	Alexandrium taylori	ITS1t 5.8S-3'	TGGTGTTTGAATGCGGTTGT GCAMACCTTCAAGMATATCCC	
ITS1–5.8S–ITS2	*Alexandrium taylori*	Tay5′ Tay3′	TGGTGTTTGAATGCGGTTGT AGGAAATGGCACCAGAATGC	Galluzzi et al., 2010
18S-ITS1–5.8S–ITS2-28S	*Alexandrium catenella*	FACAT	TGATATTGTGGGCAACTGTAA	Genovesi et al., 2011
	Alexandrium tamarense	FATAM TACATAM	TGGTAATTCTTCATTGATTACAATG AACATCTGTTAGCTCACGGAA	

ITS	*Alexandrium tamiyavanichii*	Atami-F1 Atami-R1	AAGCTTGCTGTGGGTACAGA TACAGCTCACAGCAATGCAG	Nagai, 2011
ITS	*Alexandrium affine*	Affn-F1 Affn-R2	CTTGCTTCAAGCTGGTATGTC GTCAATGTTCACCATTTCACCA	
(f) forward				
(r) reverse				

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2429 **Table 3. Probe sequenes for target ribosomal DNA genes of *Alexandrium* species**

Probe name	Target gene	Sequence (5'-3')	Specific for	Reference
AOST1	18S	CAACCCTTCCCAATAGTCAGGT	*A. ostenfeldii*	Metfies et al., 2005
AOST2	18S	GAATCACCAAGGTTCCAAGCAG	*A. ostenfeldii*	Metfies et al., 2005
AOST02	18S	CACCAAGGTTCCAAGCAG	*A. ostenfeldii*	John et al., 2003a
ALEXMIN1	18S	CCCAGAAGTCAGGTTTGGAT	*A. minutum* (AY831408, AY883006, AJ535380, AJ535388)	Nölte, unpublished
Act1	28S	GCACTTGCAGCCAAAACCCA	*A. catenella* (Temperate Asian Clade, Group IV)	Sako et al., 2004
ATNA01	28S	AGTGCAACACTCCCACCA	*A. tamarense* (North American Clade, Group I)	Miller and Scholin, 1998
Atm1	28S	ACACCCACAGCCCAAAGCTC	*A. tamarense* (North American Clade, Group I)	Sako et al., 2004
ATAM01	28S	TTCAAGGCCAAACACCTG	*A. tamarense* species complex	John et al., 2005
ATNA02	28S	AACACTCCCACCAAGCAA	*A. tamarense* (North American Clade, Group I)	John et al., 2005
ATWE03	28S	GCAACCTCAAACACATGG	*A. tamarense* (Western European Clade, Group III)	John et al., 2005
ATME04	28S	CCCCCCCACAAGAAACTT	*A. tamarense* (Mediterranean Clade, Group II)	John et al., 2005
AMINC	18S	GAAGTCAGGTTTGGATGC	*A. minutum*	Diercks et al 2008

AMINC				
NEXT	18S	TAATGACCACAACCCTTCC	*A. minutum*	
TamA	28S	TCACCCACAGCCAAAACCTA	*A. tamarense* (Western European Clade, Group III)	Touzet et al., 2010
TamToxC	28S	GCAAGTGCAACACTCCCACCA	*A. tamarense* (North American Clade, Group I)	Touzet et al., 2010

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Figure Legends

Figure 1.

Phylogenetic tree inferred by maximum likelihood analysis of partial LSU rDNA (D1-D2 domains) of 21 nominal species of *Alexandrium*. Analysis includes a subset of taxa included in the maximum likelihood phylogenetic analysis of 28S rDNA by Touzet et al. (2008a). This analysis was supplemented by additional sequences for some species (or ribotypes of species complexes) from previous phylogenetic studies: *A. pseudogonyaulax* (MacKenzie et al., 2004), *A. tropicale* and *A. minutum* 'Pacific clade' (Lilly et al., 2005), *A. ostenfeldii* (Kremp et al., 2009), *A. tamutum* (Montresor et al., 2004), *A. fraterculus* and *A. taylori* (John et al., 2003b), and *A. tropicale* and *A. tamiyavanichi* (Menezes et al., 2010). In addition, *Pyrodinium bahamense* sequences used in the analyses by Leaw et al. (2005) were included, as well as those of other gonyaulacoid dinoflagellates, to demonstrate monophyly of the genus *Alexandrium*. *Prorocentrum minimum* was set as the outgroup. Sequences were aligned with MAFFT v6.814b (Katoh and Kuma, 2002) in Geneious 5.4.4 and the TrN+G model of base substitution was determined according to the Akaike Information Criterion and the Bayesian Information Criterion as the optimal model with jModeltest (Posada, 2008). Maximum likelihood analyses were carried out with PhyML (Guindon and Gascuel 2003) in Geneious 5.4.4 with the following constraining parameters: base frequency (A= 0.26832, C= 0.15771, G= 0.25629, T= 0.31768), Transition/transversion ratio for purines: 2.267, Transition/transversion ratio for pyrimidines: 4.725, gamma distribution shape parameter (G= 0.755). Branch frequencies from 100 bootstrap replicates are given in percent at the respective nodes if >50%. The two subgenera *Alexandrium* and *Gessnerium* (light gray

shaded) do not form reciprocal monophyletic clades. Species complexes, such as the *A. tamarense* species complex, contain non-reciprocal monophyletic clades according to morphologically determined taxa, which rather resemble evolutionary units with distinct biogeographical distributions and varying degrees of morphological plasticity.

* Isolate was originally misidentified as *A. tropicale* (Lilly et al., 2007)

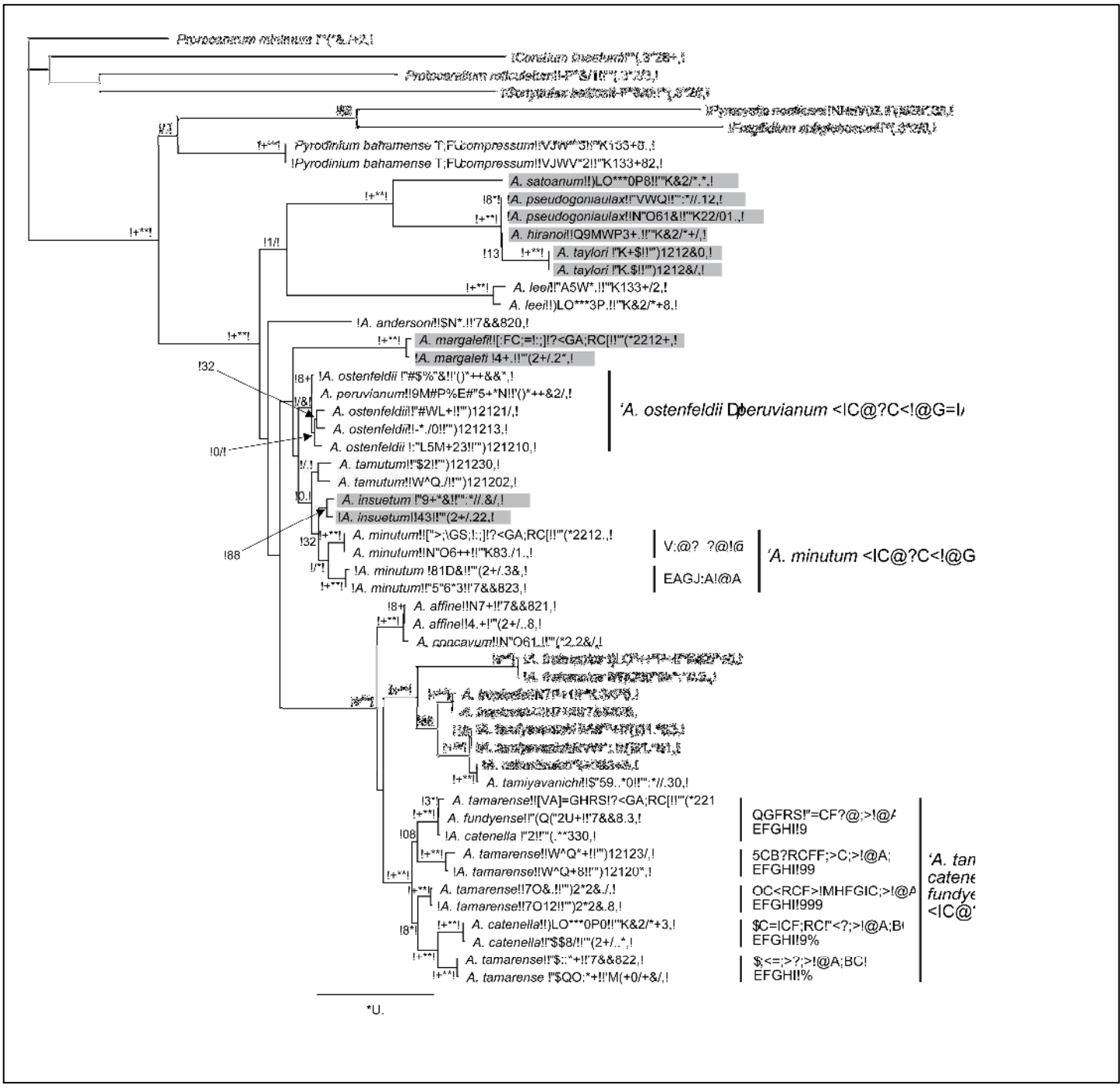
Figure 2. Distribution of *Alexandrium* species in the Mediterranean Sea, modified from Penna et al. (2008). Open circles represent the sampled stations. Colored circles, square, triangle, and diamond symbols represent the species found by Penna et al. (2008) or by other authors, as defined and based on nucleotide sequences and morphology (see Section 2.3).

Alexandrium andersoni (▲), *A. minutum* (●), *A. tamutum* (●), *A. peruvianum / A. ostenfeldii* (●), *A. insuetum* (●), *A. margalefi* (◆), *A. pseudogonyaulax* (●), *A. taylori* (●), *A. affine* (●), *A. catenella* Group VI (◆), *A. tamarense* Group II (□), and Group III (△).

Figure 3. Schematic representation of the life cycle of heterothallic *Alexandrium* species. Species have a haplontic life cycle, i.e. the motile vegetative cells (1) are haploid. Under specific conditions, usually related to stress, some vegetative cells can transform into a non-motile pellicle cyst (2) that can rapidly switch back to the motile stage when conditions improve. The sexual phase starts with the formation of gametes (3), which conjugate (4) and form a diploid planozygote (5). Depending on environmental conditions, the planozygote can transform into a resting cyst (hypnozygote (6) or, for some species, can undergo meiosis and

produce a vegetative cell (1). Cysts can spend variable periods of time in the sediments and, upon germination, release a motile cell

2480 Figure 1.



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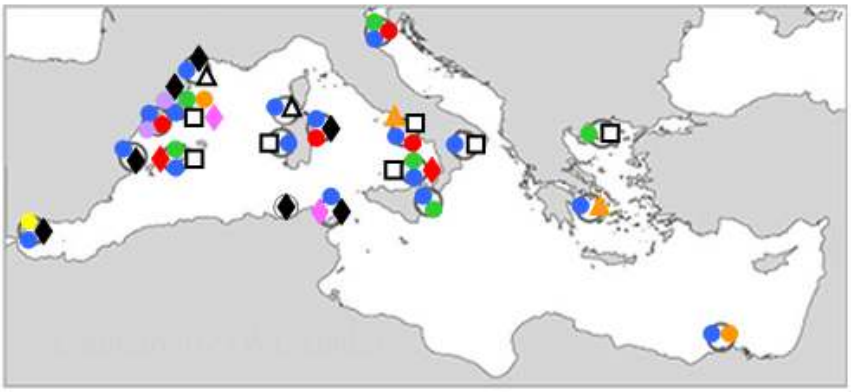
2483 Figure 2.



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2487 Figure 3.